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Acylation of Biomolecules in Prokaryotes: a Widespread Strategy for the Control of Biological Function and Metabolic Stress

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SUMMARY

Acylation of biomolecules (e.g., proteins and small molecules) is a process that occurs in cells of all domains of life and has emerged as a critical mechanism for the control of many aspects of cellular physiology, including chromatin maintenance, transcriptional regulation, primary metabolism, cell structure, and likely other cellular processes. Although this review focuses on the use of acetyl moieties to modify a protein or small molecule, it is clear that cells can use many weak organic acids (e.g., short-, medium-, and long-chain mono- and dicarboxylic aliphatics and aromatics) to modify a large suite of targets. Acetylation of biomolecules has been studied for decades within the context of histone-dependent regulation of gene expression and antibiotic resistance. It was not until the early 2000s that the connection between metabolism, physiology, and protein acetylation was reported. This was the first instance of a metabolic enzyme (acetyl coenzyme A [acetyl-CoA] synthetase) whose activity was controlled by acetylation via a regulatory system responsive to physiological cues. The above-mentioned system was comprised of an acyltransferase and a partner deacylase. Given the reversibility of the acylation process, this system is also referred to as reversible lysine acylation (RLA). A wealth of information has been obtained since the discovery of RLA in prokaryotes, and we are just beginning to visualize the extent of the impact that this regulatory system has on cell function.

INTRODUCTION

Posttranslational modifications (PTMs) are important for the regulation of protein structure and function (1). These modifications allow organisms to rapidly respond and adapt to changing environmental conditions, forgoing the need to transcribe and translate new proteins by simply modifying the function of existing proteins. Examples of PTMs include acetylation (2), glycosylation (3), lipidation (4), methylation (5), *S*-nitrosylation (6), phosphorylation (7, 8), succinylation (9), ubiquitinylation (10), adenylylation and phosphocholinylation (11), ADP-ribosylation (12), serine/threonine *O*-acetylation (13), proteolysis (14–16), and others.

Protein acylation is a broadly distributed PTM in prokaryotes. This review focuses on reversible acetyl coenzyme A (acetyl-CoA)-dependent, enzyme-driven protein acetylation, which is broadly distributed in all domains of life. Protein acetylation can occur at the N terminus on the alpha amino group (N^{α}-acetylation) or the epsilon amino moiety of lysyl side chains (N^{ε}-acetylation) (Fig. 1). Notably, N^{α}-acetylation can occur co- or posttranslationally, alters protein stability, and is typically irreversible (17, 18). In contrast, N^{ε}-acetylation can modify protein structure and function and typically is reversible by a deacetylase (Fig. 2).

Discovery of Protein Acetylation

Lysine acetylation was first reported in the 1960s as a modification of the lysine-rich N-terminal tails of eukaryotic histones (2). Histones are protein components of chromatin, the compact DNA structure in eukaryotes, and acetylation of these proteins is tightly controlled. Histone acetylation is carried out by histone acetyltransferases (HATs), and is associated with decreased DNA binding due to the loss of interactions between the epsilon amino (N^{ε}) group of lysines and the phosphate anions of the DNA strands. Acetylation of the N^{ε} group of lysines effectively neutralizes the positive charge of lysine, relaxing histone/DNA interactions and hence providing an opportunity for the transcriptional machinery to decode genes that otherwise would be unavailable (19, 20). In eukaryotes, the tumor suppressor protein p53 was the first mammalian transcription factor shown to be regulated by acetylation (21). Now, there are >100 transcription factors have been identified as acetylation targets (22).

As mentioned above, reversible lysine acylation (RLA) was discovered in prokaryotes in the early 2000s (23, 24), and this discovery led to a rapid expansion of the role of acylation, specifically acetylation, in prokaryotic cell physiology. Subsequently, RLA has been observed in bacteria, archaea, and eukaryotes (25), and it is now clear that many nonhistone proteins are also posttranslationally regulated by RLA.

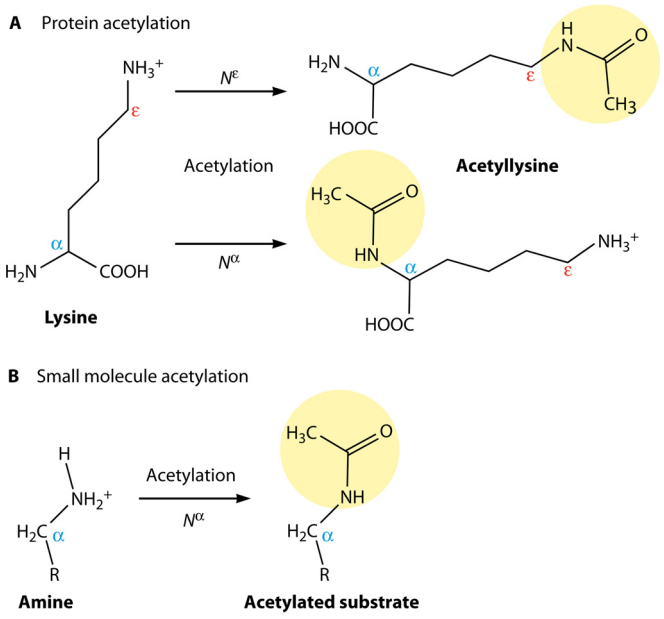

FIG 1 Schematic of N^{ε}- and N^{α}-acetylation. Protein acetylation can occur via two methods, acetylation of the ε-amino group of internal lysine residues (N^{ε}-acetylation) (red) (A) or acetylation of the N-terminal α-amino group (N^{α}-acetylation) (blue) (B). N^{ε}-Acetylation occurs posttranslationally, can be reversible, and can alter protein structure and function. N^{α}-Acetylation occurs co- or posttranslationally, is typically not reversible, and alters protein stability.

At present, RLA is known to affect the function of diverse cellular processes, including chromatin maintenance (26), regulation of gene expression (27), metabolism (28–31), and cell structure (32). RLA exerts its effects by modulating DNA binding, protein-protein interactions, enzyme activity, substrate binding, and protein stability (33, 34).

Studies have shown that a variety of proteins are regulated by RLA, including the metabolic enzymes acetyl-CoA synthetase (23) and phosphoenolpyruvate carboxykinase (35), the M2 isoform of pyruvate kinase (36), phosphoglycerate mutase 1 (37), and the structural protein α-tubulin, a subunit of microtubules (38). Acetylation has been suggested to rival phosphorylation in both its prevalence and diversity of target substrates (39).

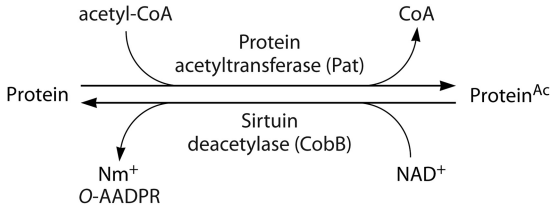

FIG 2 RLA schematic. A protein substrate (form 1) is modified by a protein lysine acetyltransferase (Pat) (of the GNAT family), resulting in the acetylated protein (form 2). This modification is reversible, either by a NAD^+-consuming class III sirtuin deacetylase, CobB, or a Zn(II)-dependent protein deacetylase. The sirtuin deacetylase uses NAD^+ as a substrate and not as a coenzyme. Sirtuins modify the carboxyl group of the ribose of the NMN moiety of NAD^+, simultaneously releasing nicotinamide (Nm). The resulting by-product is *O*-acetyl-ADP-ribose (*O*-AADPR).

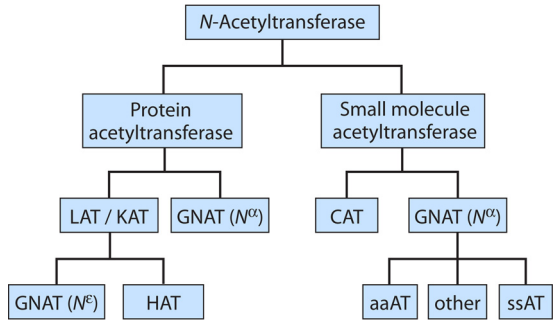

FIG 3 Acyltransferase nomenclature and classification. Abbreviations: LAT/KAT, lysine (K) acetyltransferase; GNAT, Gcn5 *N*-acetyltransferase; HAT, histone acetyltransferase; CAT, chloramphenicol acetyltransferase; aaAT, arylamine acetyltransferase; ssAT, spermine/sperimidine acetyltransferase; Other, unclassified acetyltransferase; N^{ε}, acetylation of the epsilon amino group of a lysine; N^{α}, acetylation of the alpha amino group of any N-terminal amino acid.

LYSINE ACETYLTRANSFERASES

Diversity

There are three classes of lysine (Lys [K]) acetyltransferases (LATs) (also known as KATs) that catalyze the transfer of the acetyl moiety from acetyl-CoA to the ε-amino group of lysine side chains (Fig. 3). These classes of LATs comprise a large and diverse set of enzymes named after their founding member(s), including (i) the Gcn5-related *N*-acetyltransferase (GNAT) family (named after the yeast Gcn5 protein [Pfam 00583]), (ii) the MYST family (named after human MOZ, yeast Ybf2/Sas3, yeast Sas2, and human Tip60 [Pfam 01853]), and (iii) the p300/CBP family (named after human hp300 and hCBP [Pfam 06466]). The above-mentioned enzyme families differ in sequence similarity, domain organization, substrate specificity, and catalytic mechanism (40–48). The MYST and p300/CBP families are present only in eukaryotes, while the GNAT family is present in all domains of life.

Conservation of the Acetyl-CoA Binding Domain

Regardless of their mechanistic differences, acetyltransferases contain a conserved core domain which binds to acetyl-CoA through interactions with the pyrophosphate and pantothenate moieties (41, 47, 49–52; reviewed in references 42 and 53). Neither the adenine base nor the acetyl moiety significantly contributes to binding the core domain. Due to this binding mode, acetyltransferases bind acetyl-CoA with high affinity but low specificity (54, 55), allowing for recognition of various acyl-CoA thioesters as well as free coenzyme A (CoASH). Their specificity is then due to their structurally divergent N- and C-terminal domains outside the core domain (49). In some cases, other acyl-CoA thioesters, such as propionyl-CoA and succinyl-CoA, are physiologically relevant substrates (43, 56, 57) and are discussed in more detail below.

Mechanisms of acetylation. The mechanism of transfer of acyl moieties used by the GNAT and MYST families involves a catalytic glutamate residue that acts as a general base, facilitating a water-mediated proton abstraction from the side chain of the substrate lysine (45, 49, 53, 58) (Fig. 4). The lysine amine group initiates a nucleophilic attack on the carbonyl carbon of the acetyl moiety of CoA, allowing the direct transfer of the acyl group to the substrate lysine. Members of the MYST family employ either a catalytic mechanism similar to that of GNATs or a ping-pong mechanism

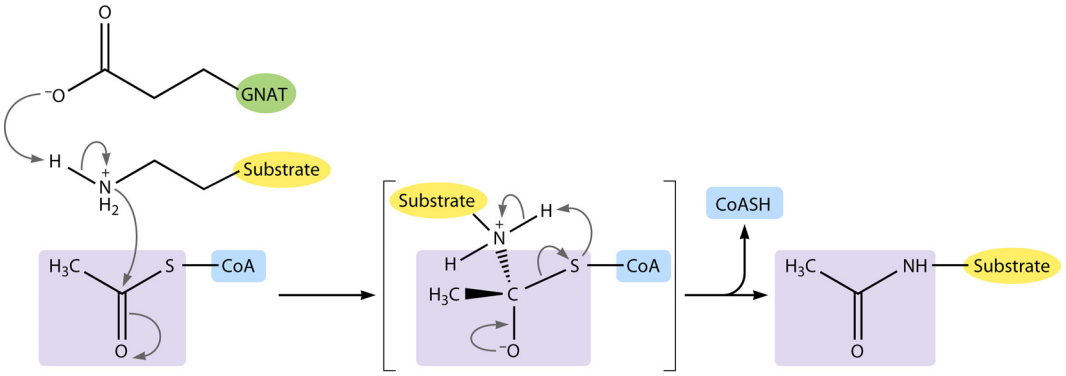

FIG 4 Acetylation mechanism of GNATs. The GNAT acetylation mechanism involves a catalytic glutamate that acts a general base, facilitating a water-mediated proton abstraction from the side chain of the substrate lysine. The ε-amino group of lysine performs a nucleophilic attack on the carbonyl carbon of the acetyl moiety of CoA, allowing direct transfer of the acetyl group to the lysine side chain.

(also known as a double-displacement mechanism) involving an acetylated enzyme intermediate (46). Members of the p300/CBP family are structurally distinct and do not use a catalytic base to initiate the transfer of the acyl moiety (47). Instead, it appears that the p300/CBP family uses a Theorell-Chance mechanism, a sequential mechanism that does not form a stable ternary complex (47).

Bacterial Gcn5-Related *N*-Acyltransferases

The GNAT family is conserved among archaea, bacteria, and eukaryotes (24, 53, 59). All known bacterial lysine acetyltransferases identified to date belong to the GNAT superfamily (60). Despite having low sequence homology, GNATs share a conserved catalytic fold and can acetylate both protein and small-molecule substrates (53). The first bacterial GNATs were characterized as aminoglycoside *N*-acetyltransferases from *Enterococcus faecium* (61) and *Serratia marcescens* (62), demonstrating that GNATs acetylate diverse substrates, ranging from histones to antibiotics (2, 62, 63).

Utilization of alternative acyl-CoA substrates. Modifications of the N^{ε} amino group of lysine by propionyl, malonyl, succinyl, and butyryl moieties have also been demonstrated for metabolic enzymes, transcription factors, and histones in both bacteria and eukaryotes (56–58, 64, 65). For example, in *Salmonella enterica*, the activity of propionyl-CoA synthetase (PrpE) is controlled by propionylation (56). Both lysine propionylation and butyrylation have also been identified as reversible modifications that occur on histones (57, 66), expanding the range of GNAT-mediated regulation through their ability to utilize various acyl-CoA substrates.

Overview of GNAT structures. GNATs comprise one of the largest enzyme superfamilies identified thus far (>10,000 members), and dozens of GNAT structures have been resolved and are available from the RCSB Protein Data Bank (PDB) (http://www.rcsb.org/pdb/home/home.do). Despite having low to moderate primary sequence homology, GNATs contain a core catalytic domain that is structurally well conserved (Fig. 5A and B). The GNAT domain contains a central β-sheet (six antiparallel strains) composed of four distinct motifs, motifs A (β4,α3), B (β5,α4), C (β1,α1-2), and D (β2-3), which were originally identified by sequence similarity (67). Motif A has the highest conservation and is important for acetyl-CoA binding and catalysis (reviewed in references 53 and 54).

Abundance and distribution of GNATs. The number of GNATs present in a given organism varies, with the majority of commonly studied organisms encoding ~20 to 25 GNATs. As an example of the diversity and abundance of GNATs, the Gram-positive, intracellular pathogen *Listeria monocytogenes* encodes ~14 GNATs, the purple nonsulfur alphaproteobacterium *Rhodopseudomonas palustris* encodes ~26 GNATs, and the Gram-positive actinomycete *Streptomyces lividans* encodes ~72 putative GNATs. The majority of these GNATs are uncharacterized, with no known function. See Table 1 for an overview of the number of GNATs present in commonly studied prokaryotes. The sheer prevalence of GNATs raises many questions regarding the physiological role and substrate specificity of these enzymes. It could be speculated that the range of GNATs that an organism encodes may be driven by the diversity of the environments inhabited.

Diversity of domain organization of GNAT protein acetyltransferases. Protein acetyltransferases of the GNAT family exhibit a diverse domain architecture, four of which have been studied and are discussed (Fig. 6).

The *S. enterica* Pat (*Se*Pat) enzyme, the first enzyme of type I to be discovered (24), has homologues in *Escherichia coli* (*Ec*Pka), *R. palustris* (*Rp*Pat), *Vibrio* species, and cyanobacteria, among others. These *Se*Pat homologues are comprised of two distinct domains, a large (~700-residue) N-terminal domain and the catalytic GNAT domain (~200 residues) at the C terminus (68, 69). Although the function of the large N-terminal domain of Pat enzymes remains largely unclear, insights into its relevance to Pat function have been reported (70, 71). Briefly, results obtained from *in vitro* and *in vivo* analyses of single-amino-acid *Se*Pat variants showed that such variants had low enzymatic activity (70). Furthermore, results from recently reported structural work aimed at understanding the substrate specificity of Pat enzymes suggested that in the absence of the large domain of Pat, the catalytic domain of the enzyme inefficiently interacts with its protein substrate (71). Whatever the role of the N-terminal domain may be, it is likely to also play a role in sensing acetyl-CoA. This conclusion was drawn on the basis of results from isothermal calorimetry experiments, which showed that *Se*Pat binds two molecules of acetyl-CoA: one binds to the N-terminal domain, and the other one binds to the catalytic domain (70, 72–77).

Streptomyces lividans encodes a type II protein acetyltransferase (*Sl*PatA), in which the domain order is reversed relative to

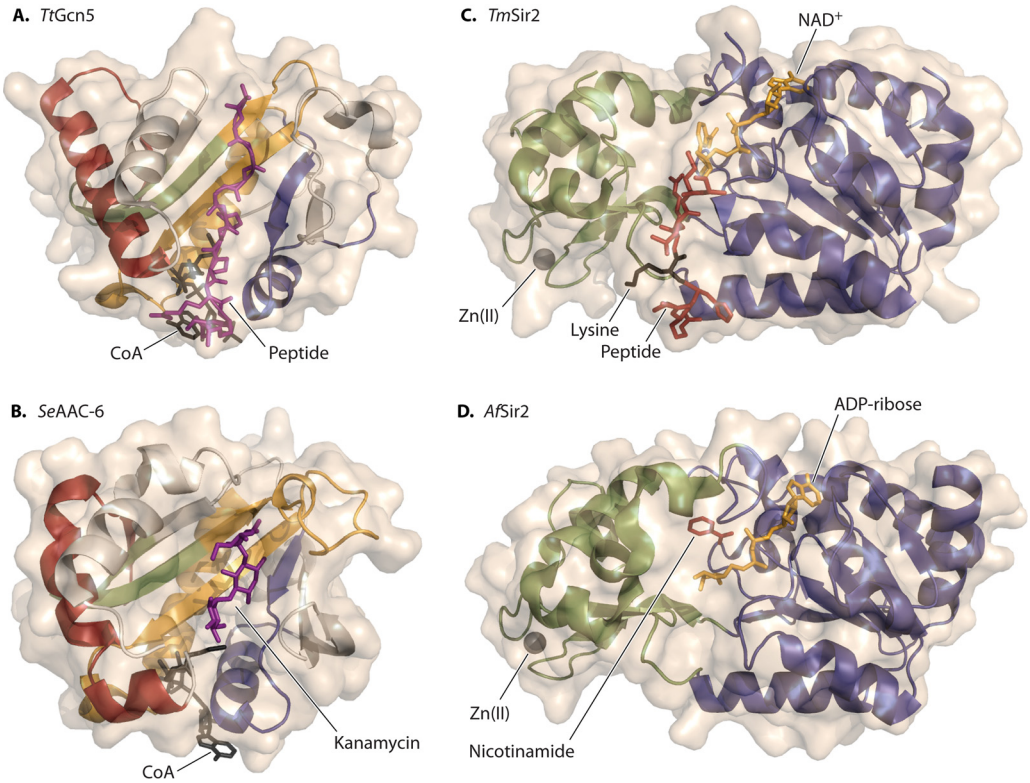

FIG 5 GNAT and sirtuin structural overview. GNAT domains are comprised of a central β-sheet and contain four motifs (motifs A [gold], B [blue], C [red], and D [green]). (A and B) Examples of GNAT structures. (A) *Tt*Gcn5 is shown with an H3 11-mer peptide substrate (purple sticks). (B) *Se*AAC(6′) is shown in complex with its substrate kanamycin (purple sticks), and CoA is shown in black sticks. (C and D) Examples of sirtuin structures. These enzymes contain a Rossmann fold domain (blue) and a variable Zn(II) binding domain (green) [with Zn(II) shown as a gray sphere]. The binding sites for NAD^+ (gold) and the acetyllysine substrate (red sticks, with lysine in black sticks) are located in a cleft between the two domains (C). The products of the reaction, nicotinamide (red sticks) and ADP-ribose (gold sticks) are shown (D). (C) *Thermotoga maritima* Sir2 (*Tm*Sir2) is shown with a peptide substrate (red sticks). (D) *Af*Sir2 from the archaeon *Archaeoglobus fulgidus* is shown. PDB accession numbers are as follows: 1QSN (A), 2QIR (B), 2H4F (C), and 1YC2 (D).

the domain order observed in *Se*Pat, *Ec*Pka, and *Rp*PatA; that is, in *Sl*PatA, the GNAT catalytic domain is located at the N terminus, and the large domain of unknown function is located at the C terminus (31). Other members of the actinomycetes, as well as the archaeon *Archaeoglobus fulgidus*, exhibit the same domain organization. Notably, the *Sl*PatA large domain contains a proline-rich region that includes a degenerate collagen-like GPS motif. The role of the degenerate collagen-like GPS has not been established, and the presence of this additional feature of *Sl*PatA may suggest that the regulation of this enzyme is more complicated than the regulation other Pat homologues.

The large domains of type I and type II Pat enzymes share homology with ADP-forming acyl-CoA synthetases (Pfam 13380) that catalyze the following reaction: free acid + ATP + CoA $\rightleftharpoons$ acyl-CoA + ADP + P_i. ADP-forming acyl-CoA synthetases have been characterized in archaea and protists (75–77). In spite of this homology, no catalytic activity has been attributed to the N-terminal domain of type I or type II GNATs (73, 74).

Mycobacterium tuberculosis and *Mycobacterium smegmatis* each encode a type III two-domain protein acetyltransferase (*Mt*PatA and *Ms*PatA, respectively) in which the C-terminal GNAT domain is fused to an N-terminal cyclic AMP (cAMP) binding regulatory domain (discussed in more detail below).

Several prokaryotes encoding single-domain type IV GNAT protein acetyltransferases have been characterized. These enzymes are substantially smaller (~200 residues) than the large two-domain Pat homologues (~800 to 1,100 residues), yet they appear to perform similar functions. Single-domain GNATs have been identified and characterized in *Bacillus subtilis* (*Bs*AcuA) (30, 78), *R. palustris* (*Rp*KatA) (28), and the archaeon *Sulfolobus solfataricus* (*Ss*Pat) (79). The range of domain architectures and organizations in the bacterial and archaeal GNATs reveals that lysine acetylation is most likely regulated by diverse signals within these organisms.

A word of caution about the nomenclature of Pat enzymes. The reader should be cautioned about the "Pat" abbreviation being used to name acetyltransferases that do not belong to the type I, type II, or type III protein acetyltransferases. As a case in point, the Pat enzyme from *Sulfolobus solfataricus* (*Ss*Pat) (PDB accession number 3F8K) is only 160 residues long (73, 74). Such a length is substantially shorter than the typical length of type I Pat enzymes, which are between 850 and 1,100 residues long. The *Ss*Pat enzyme actually is a type IV lysine acetyltransferase.

Known GNAT functions in *Escherichia coli* and *Salmonella enterica*. The model organisms *E. coli* and *S. enterica* encode ~26 GNAT homologues, only half of which have known or predicted functions (Table 2). These GNATs target primary amines (80, 81), including the N termini of proteins (82, 83), aminoglycoside antibiotics (63), polyamines (84), a nucleotide sugar (85), glutamate (86), toxic aminoacyl nucleotides (87), and transfer RNAs (88).

TABLE 1 Representative frequency of prokaryotic RLA components

Organism	No. of RLA components present		
	GNATs	Sirtuins	HDACs
Archaea			
Methanococcus maripaludis	5	0	0
Pyrococcus furiosus	7	1	1
Sulfolobus solfataricus	8	1	3
Bacteria			
Actinobacteria			
Mycobacterium tuberculosis	19	1	0
Streptomyces coelicolor	72	2	1
Bacteriodetes			
Bacteroides thetaiotaomicron	8	1	0
Deinococcus-Thermus			
Thermus thermophilus	9	1	3
Firmicutes			
Bacillus subtilis	28	1	1
Clostridium difficile	24	1	0
Geobacillus kaustophilus	9	2	0
Lactobacillus casei	32	1	0
Listeria monocytogenes	14	1	0
Staphylococcus aureus	29	1	1
Streptococcus pneumoniae	22	0	0
Alphaproteobacteria			
Caulobacter crescentus	26	1	3
Rhodopseudomonas palustris	24	1	1
Ruegeria sp. strain TM1040	25	1	2
Betaproteobacteria			
Bordetella pertussis	12	1	1
Neisseria meningitidis	7	0	1
Deltaproteobacteria-Epsilonproteobacteria			
Campylobacter jejuni	4	1	0
Helicobacter pylori	11	1	0
Myxococcus xanthus	47	1	3
Gammaproteobacteria			
Erwinia amylovora	19	1	0
Escherichia coli	26	1	0
Francisella tularensis	5	0	0
Haemophilus influenzae	3	2	0
Legionella pneumophila	18	0	0
Proteus mirabilis	13	1	0
Pseudomonas aeruginosa	22	2	0
Salmonella enterica	26	1	0
Shigella flexneri	21	1	0
Vibrio cholerae	31	1	2
Yersinia pestis	14	1	0
Spirochaetes			
Borrelia burgdorferi	1	0	0

Three GNAT enzymes, RimI, RimJ, and RimL, acetylate the α-amine group at the N terminus of the ribosomal proteins S18, S5, and L12, respectively (89, 90). The *Ec*YncA homologue of *S. enterica* (*Se*MddA) was recently shown to acetylate and detoxify oxidized methionine derivatives (e.g., methionine sulfoximine and methionine sulfone) (91). Pka is the only identified protein lysine acetyltransferase (Pat in *S. enterica*) (24, 92) (discussed in more detail below). Two other *E. coli* GNATs appear to modify proteins through unusual mechanisms. Aat is a leucyl, phenylalanyl-tRNA-protein transferase that modifies proteins targeted for degradation through the N-end rule degradation pathway by the attachment of a leucine or phenylalanine to lysyl and arginyl residues (93, 94). In *S. enterica*, a GNAT homologue known as PanM (formerly YhhK) promotes cleavage and maturation of the L-aspartate-α-decarboxylase zymogen (pro-PanD), an enzyme of the coenzyme A biosynthetic pathway (14, 15). PanZ, the PanM homologue in *E. coli*, performs the same function in this bacterium (95, 96).

LYSINE DEACETYLASES

N-Lysine protein acetylation is reversible by protein deacetylases, which, for historical reasons, are referred to as histone deacetylases (HDACs) (Pfam 08295). Members of four HDAC families catalyze *N*-lysine deacylation. The related class I, II, and IV HDACs do not require cofactors and catalyze lysine deacylation via hydrolysis of the acyl group (Pfam 08295) (55, 97) (Fig. 7A). In contrast, class III HDACs, commonly referred to as sirtuins (Pfam 02416), are a mechanistically distinct family of NAD^+-dependent deacetylases (97, 98) (Fig. 7B). The name sirtuin refers to any protein that is homologous to the yeast SIR2 protein. Bacteria and archaea typically encode one to two sirtuin homologues, whereas eukaryotes encode several, with human cells encoding seven (59, 99) (Table 1).

Zinc-Dependent Histone (Lysine) Deacetylases

HDACs belonging to class I, II, and IV families also deacetylate nonhistone protein substrates and therefore are referred to as lysine deacetylases (reviewed in references 100 and 101). The core feature of the HDAC structure is an α/β deacetylase fold comprised of an 8-stranded parallel β-sheet in which conserved residues coordinate a Zn(II) ion required for catalysis. Catalysis is mediated by a histidinyl residue, which acts as a general base and, in conjunction with the Zn(II) ion, activates a metal-bound water molecule that triggers a nucleophilic attack on a carbonyl group of the substrate. HDACs are unique in their ability to catalyze the deacetylation of both protein and small-molecule substrates (Fig. 7). *R. palustris* (LdaA) and *B. subtilis* (AcuC) proteins are examples of bacterial HDACs that deacetylate proteins and are discussed below.

NAD^+-Dependent Sirtuin Deacetylases

Although described as deacetylases, some sirtuins have depropionylase activity (56, 57, 102). In eukaryotes, certain sirtuins have also been shown to have protein desuccinylation (103, 104) and demalonylation (105) activities and are thought to play a critical role in mitochondrial metabolism (reviewed in references 106 and 107). The ability of sirtuins to deacylate a variety of modifications correlates with the ability of their partner GNATs to utilize alternative acyl-CoA donors. This provides a mechanism with a wider range of modifications available to the cell to maintain homeostasis under diverse physiological conditions in response to environmental changes.

Sirtuins hydrolyze NAD^+. Due to their requirement for NAD^+, sirtuin deacetylases have garnered a great deal of interest for their ability to "sense" and respond to NAD^+ levels, which in turn reflect the cellular energy status (108, 109). Deacetylation is an energetically favorable process and can be catalyzed by the class I, II, and IV deacetylases without cofactors. It is therefore of interest that sirtuins (class III) hydrolyze NAD^+ in the course of the deacetylation reaction, an essential metabolic cofactor (110, 111). The resynthesis of NAD^+ from the hydrolysis products requires

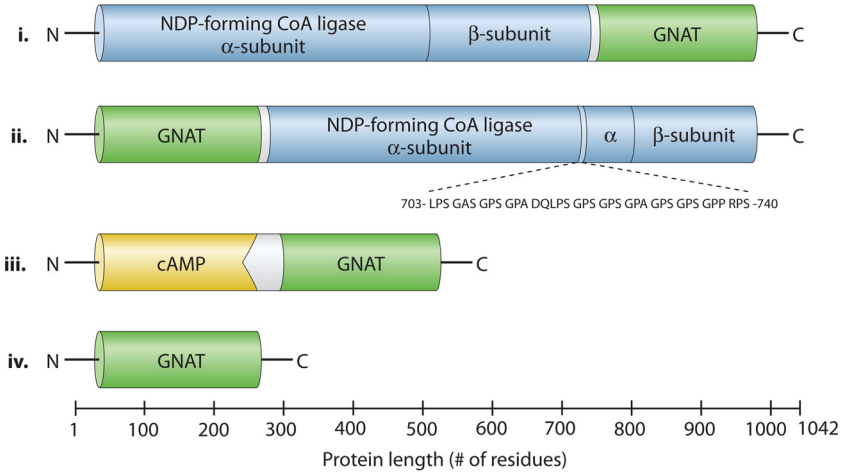

FIG 6 Diversity in the domain organization of prokaryotic protein acetyltransferases. GNAT protein acetyltransferases characterized to date exhibit different domain organizations: an N-terminal domain of unknown function homologous to an ADP-forming acyl-CoA synthetase domain fused to a C-terminal GNAT domain (type I); an N-terminal GNAT domain fused to a C-terminal domain of unknown function homologous to an ADP-forming acyl-CoA synthetase domain, which contains a GPS motif, a 37-aa-long, degenerate proline-rich domain typically found in collagen (250) (type II); a GNAT domain fused to an N-terminal cAMP binding domain (type III); and a single GNAT domain (type IV).

~8.2 kcal/mol of energy and is energetically expensive (110, 112). It stands to reason that there is a compelling reason to tie NAD^+ levels to sirtuin activity (i.e., protein acetylation state) (109).

Sirtuin reaction mechanism. Sirtuin deacetylases have an unusual catalytic mechanism that uses NAD^+ not as a cofactor but as a cosubstrate that is cleaved during the deacylation reaction, yielding *O*-acyl-ADP-ribose (*O*-AADPR) (97, 113–116). Sirtuin-catalyzed deacetylation is initiated by the binding of NAD^+ to the catalytic site. The formation of an imidate intermediate occurs through one-step ADP-ribosylation and an inversion of configuration (reviewed in reference 117) (Fig. 7). The inversion of configuration was predicted by the original discovery that sirtuins were enzymes that used pyridine nucleotides as substrates (98). The reaction of the coenzyme B_{12} biosynthetic pathway in *S. enterica* that is also performed by the CobB sirtuin proceeds via a nucleophilic attack inverting the configuration of the *N*-glycosidic bond between the base and the ribosyl moiety of the pyridine nucleotide cosubstrate (117). The products formed from the deacetylation reaction are (i) the deacylated protein, (ii) nicotinamide, and (iii) *O*-acyl-ADP-ribose. The *O*-acyl-ADP-ribose byproduct is generated through the mono-ADP-ribosylation of the removed acyl group (102, 116, 118, 119).

TABLE 2 Roles of *E. coli* Gcn5 *N*-acetyltransferases

Protein	Function	Reference(s)
Aat	Leucyl, phenylalanyl-tRNA-protein transferase	93
ArgA	Glutamate acetyltransferase	86
PanM/PanZ	PanD maturation factor	14
PhnO	Aminoalkylphosphonic acid acetyltransferase	251
Pka/YfiQ/Pat	*N*-Lysine protein acetyltransferase	24
RimI	S18 ribosomal protein acetyltransferase	89
RimJ	S5 ribosomal protein acetyltransferase	89
RimL	L12 ribosomal protein acetyltransferase	90
SpeG	Spermidine acetyltransferase	84
TmcA	$tRNA^{Met}$ cytidine acetyltransferase	88
WecD	dTDP-fucosamine acetyltransferase	85
YhhY	Aminoacyl nucleotide acetyltransferase	87
YncA/MnaT	Putative methionine sulfoximine/sulfone acetyltransferase	91, 252
YafP	Possible nitroaromatic compound acetyltransferase	253

Physiological importance of *O*-acetyl-ADP-ribose. The physiological role of *O*-AADPR in prokaryotes is unknown, but some information about its metabolism in eukaryotic systems has been reported. In eukaryotes, *O*-AADPR may act as a signaling molecule and may regulate gene silencing, ion channel gating, and redox regulation (118, 120). There are several eukaryotic enzymes identified as utilizing *O*-AADPR as a substrate, including two NUDIX (nucleoside diphosphate linked to X) hydrolases (Ysa1 from yeast and NudT5 from mouse) (121), ADP-ribosyl hydrolase (ARH3 from human) (122), and two enzymes with uncharacterized activities, including an esterase and a nuclear acetyltransferase (from yeast and human) (121). These enzymes utilize *O*-AADPR in various ways, generating *O*-acetyl-ribose-phosphate and AMP (Ysa1 and NudT5), acetate and ADP-ribose (ARH3; esterase), or an unknown acetylated product and ADP-ribose (nuclear acetyltransferase). More work is needed to elucidate the role of *O*-AADPR in prokaryotic physiology.

Effect of the NAD^+/NADH ratio on sirtuin function. Both NADH and nicotinamide have been reported to inhibit sirtuin function and may play a role in the regulation of sirtuin activity (123–125). Nicotinamide condenses with an ADP-ribose (ADPr)-like intermediate formed during the deacetylation reaction, which prevents the reaction from moving forward (109) and inhibits sirtuin activity noncompetitively at concentrations consistent with physiological nicotinamide levels (30 to 200 μM) (113, 126–130).

In contrast, NADH competitively inhibits sirtuin activity, with reported K_i values in the submillimolar-to-millimolar range, consistent with physiological NADH levels (97, 108, 123, 131, 132). Sirtuin activity varies with the NAD^+/NADH ratio. A high NAD^+/NADH ratio indicates that the cell is efficiently oxidizing NADH back to NAD^+, implying, among other things, that a

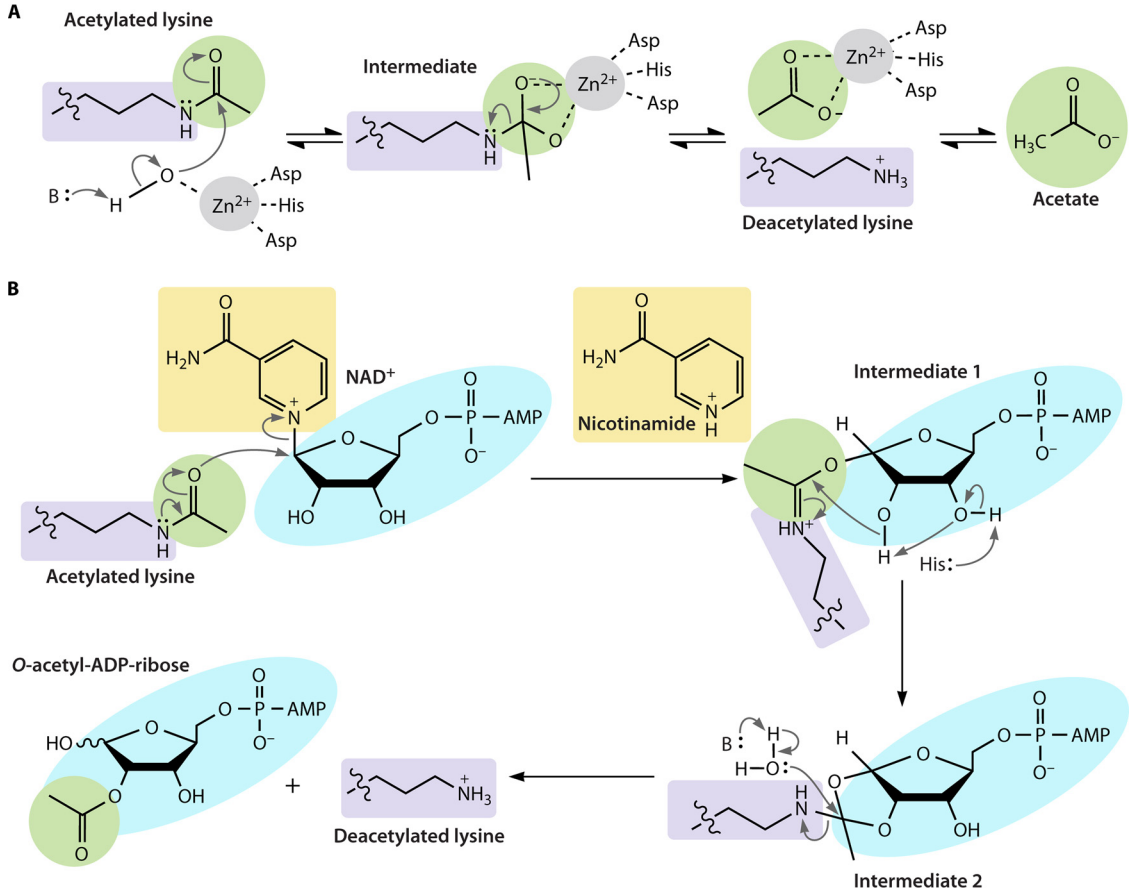

FIG 7 Deacetylation mechanisms of HDACs and sirtuins. (A) HDAC-mediated catalysis is mediated by a histidinyl residue, which acts as a general base and, in conjunction with the Zn(II) ion, activates a metal-bound water molecule for nucleophilic attack of the substrate carbonyl. The products of the HDAC reaction are deacetylated protein and acetate. (B) Sirtuin-catalyzed deacetylation is initiated by binding of NAD^+ to the catalytic site. The formation of an imidate intermediate occurs through one-step ADP-ribosylation and inversion of the configuration. The products of the sirtuin reaction are the deacylated protein, nicotinamide, and an *O*-acyl-ADP-ribose product that is derived by mono-ADP-ribosylation of the removed acyl group.

strong proton motive force is being generated under such conditions. A strong proton motive force results in high ATP levels and a concomitant increase in the demand for acetyl-CoA for anabolic purposes. If, under such conditions, acetate is present in the environment, the cell can activate it to acetyl-CoA by using the acetyl-CoA synthetase (Acs), an enzyme known to be under RLA control (discussed further below) (23). Acetylated Acs is inactive; hence, the ratio of acetylated (inactive) to deacetylated (active) Acs would be expected to be low when the NAD^+/NADH ratio is high, since NAD^+ would be available for sirtuin to deacetylate (i.e., activate) acetylated Acs (132). The NAD^+/NADH ratio is known to fluctuate with metabolism; thus, changes in this pool of free NAD^+ likely regulate the protein acylation state, with deacylation occurring when the NAD^+/NADH ratio is high and acetylation being favored when the NAD^+/NADH ratio is low.

Overview of sirtuin structures. Three-dimensional crystal structures of sirtuins from all domains of life are available (e.g., *Archaeoglobus fulgidus* [PDB accession number 1YC2]; *Thermotoga maritima* [PDB accession number 4BUZ]; *E. coli* [PDB accession number 1S5P]; *Saccharomyces cerevisiae* [PDB accession number 2HJH]; and human SIRT1 [PDB accession number 4KXQ], SIRT2 [PDB accession number 3ZGO], SIRT3 [PDB accession number 4BN4], SIRT5 [PDB accession number 3RIY], and SIRT6 [PDB accession number 3PKI]) (Fig. 5C and D). Sirtuins are comprised of a catalytic domain that contains a Rossmann fold domain and a variable Zn(II) binding domain, with divergent N- and C-terminal regions (reviewed in references 133 and 134). The Zn(II) ion in sirtuins is structurally important and does not contribute to catalysis (135). The binding sites for the nicotinamide and ribose moieties of NAD^+ and the acetyllysine substrate are located in the cleft between the large (Rossmann fold) and small [Zn(II) binding] domains (Fig. 5C and D). This binding cleft allows for substrate selectivity among different sirtuins. Importantly, NAD^+ is oriented opposite to the typical orientation seen with Rossmann fold-containing enzymes, in which the nicotinamide moiety binds to the N-terminal half of the β-sheet and the adenine binds to the C-terminal half. This orientation reversal ensures the elimination of nicotinamide.

HIGH-THROUGHPUT IDENTIFICATION OF ACETYLATED PROTEINS

Global Approaches for Identification of the Total Acetylated Protein Population

Characterization of the total acetylated protein population (referred to as the "acetylome") in a given organism has been accel-

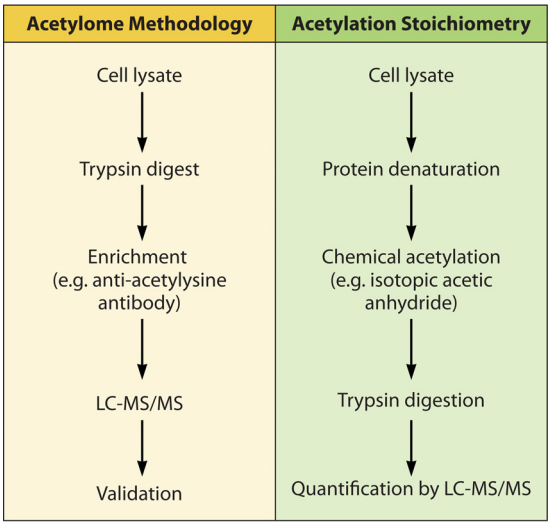

FIG 8 Methods for analysis of acetylomes. (Left column) Representative workflow of the methodology typically used to determine total acetylated protein from an organism. (Right column) Representative workflow of a recently described method to determine the level of acetylation of identified acetylated target proteins. LC-MS/MS, liquid chromatography-tandem mass spectrometry.

erated by the development of sensitive mass spectrometry-based methods that detect the precise location of acetylated lysine residues within any given protein pool (33). Such approaches have putatively identified a large number of acetylation targets, many of which appear to be acetylated at several sites. Early studies combined the use of two-dimensional separation of proteins followed by detection of acetyllysine residues by immunoblotting (136). Subsequent studies characterizing the acetylomes of eukaryotes used immunoprecipitation as a way to enrich for acetyllysine peptides present in tryptic digests of a protein pool (137–139). The combined use of anti-acetyllysine antibodies and mass spectrometry is the foundation for the current proteomic methodology used to identify acetylated peptides and proteins (Fig. 8A). These global studies have been extended to archaea (N-terminal acetylation) (140), bacteria (141–143), plants (144), parasites (145, 146), and humans (147), helping cement the fundamental principles and contributions of acetylation to cellular physiology. Such global studies consistently suggest that acetylation controls diverse cellular processes, including metabolism, transcription, translation, and cell structure.

Alternative global approaches for analysis of acetylomes. Proteome microarrays provide an alternate method for identifying the targets of acetyltransferases as well as other modifying enzymes. Protein chip technology was first reported for the analysis of protein kinases in yeast (148). Lin et al. used a similar approach to identify 13 substrates of the NuA4 acetyltransferase, including phosphoenolpyruvate carboxykinase (Pck1p) (35). This same technology was used in another study to construct a microarray comprised of *E. coli* proteins. These microarrays were probed with the *S. enterica* bacterial protein acetyltransferase (*Se*Pat) enzyme, resulting in the identification of seven substrates, including several known transcription factors (149).

Other studies have taken advantage of labeling strategies such as stable isotope labeling with amino acids in cell culture (SILAC) quantitative mass spectrometry (150). SILAC allows the detection of differences in protein abundance by using the *in vivo* incorporation of nonradioactive isotope labels. Benefits of this analysis are the identification and quantification of relative differential changes in complex protein samples.

Recent approaches have begun to tackle the problem of the stoichiometry of the level acetylation at individual sites in order to understand the biological significance of acetylation events (151, 152). The first unbiased method for determining site-specific stoichiometry of acetylated peptides using no immunoenrichment was used to investigate the acetylome of *E. coli* (151). This was achieved by chemically acetylating unmodified lysine residues by using acetic anhydride labeled with stable isotopes to generate an acetyllysine pair, which was analyzed by mass spectrometry (Fig. 8B). In this scenario, proteins acetylated endogenously contain "light" acetyllysine residues, while proteins modified chemically contain "heavy" acetyllysine residues. After resolution by mass spectrometry, the stoichiometry is determined by examining the ratio of light to heavy peak areas. By this method, those authors identified that proteins that use or generate acetyl-CoA, and those involved in transcription and translation, are the most highly acetylated (151).

Bacterial Acetylome Studies

Bacterial acetylomes have been characterized in *E. coli* (141, 142), *S. enterica* (153), *Bacillus subtilis* (154), *Erwinia amylovora* (155), *R. palustris* (28), *Staphylococcus aureus* (156), *Geobacillus kaustophilus* (157), *Vibrio parahaemolyticus* (158), *Thermus thermophilus* (159), *M. tuberculosis* (143), *Mycoplasma pneumoniae* (160), and *Streptomyces roseosporus* (161). These studies have identified a range of 62 to 667 putatively acetylated proteins per organism, with the majority of acetylated proteins being involved in central metabolism and translation (141, 156, 158) (Fig. 9).

The large number of putative acetylation targets detected by mass spectrometry has raised important questions as to the significance of the detected acetylation events and how they contribute to cellular physiology. Specifically, it is important to know how these acetylation events occur, the frequency at which they occur, and whether they affect protein function or stability. Two independent studies of acetylated proteins in *E. coli* identified 85 and 91 putative acetylation targets (141, 142). Surprisingly, only six proteins acetylated at the same lysine residue were identified in both studies (60). Since then, a third acetylome study performed in *E. coli* identified 349 acetylated proteins (162). In the related enterobacterium *S. enterica*, Wang et al. found 191 putatively acetylated proteins, many of which were metabolic enzymes (153). Although experimentally validating some of the results *in vitro*, studies by other groups were not able to reproduce these findings (28).

A combination of mass spectrometry analysis, *in vivo* genetic analyses, and *in vitro* validation for the Gram-negative photosynthetic bacterium *R. palustris* yielded the most comprehensive list of bona fide acetylation targets to date (28). In this study, acetylated proteins were identified by tandem mass spectrometry by comparing the acetylome of a wild-type *R. palustris* strain to the acetylomes of strains in which one or more acetyltransferases were absent. Stringent cutoffs were applied to reduce noise by using two different algorithms. Proteins identified in this comparison were validated by both *in vitro* and *in vivo* methods, and it was confirmed that acetylation altered the activity of each of target protein.

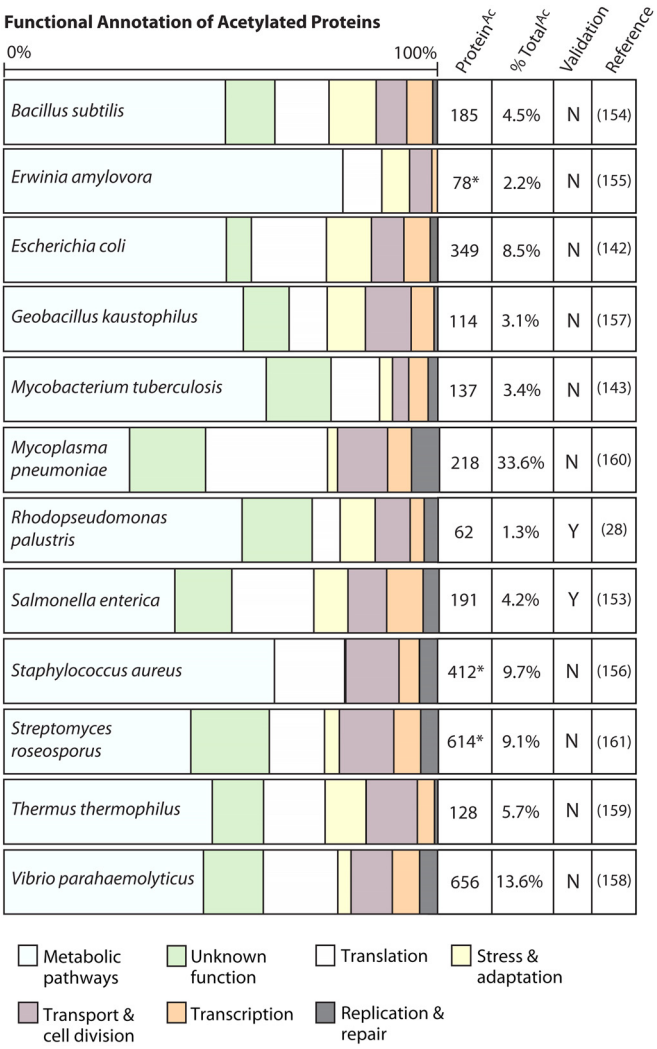

FIG 9 Comprehensive overview of bacterial acetylome studies. Shown are functional annotations of acetylated proteins identified from bacterial acetylome studies. Protein^{Ac}, number of identified acetylated proteins; % Total^{Ac}, percentage of acetylated proteins of the entire proteome; N, no; Y, yes. *, values are different than those previously reported; however, the values listed reflect the available data that were obtained from supplementary information from the cited references (28, 142, 143, 153–161). The percent scale at the top should be used to estimate the percentage of acetylated proteins in each of the categories within any given microorganism shown.

Current noteworthy issues in the field. Bioinformatics analyses reveal that protein acetyltransferases are conserved in nearly all reported genomes. This suggests that acetylation is widespread in prokaryotes and eukaryotes, which in turn implies that protein acetylation is not limited to the regulation of proteins involved in DNA maintenance or transcription. The acetylome studies provide a framework for the identification of putative targets of acetylation, but detailed mechanistic studies are needed to validate these proteomics-based results and to demonstrate that the findings are biologically relevant. There are several issues that need to be taken into consideration, which are discussed below.

(i) Global, nonenzymatic acetylation by acetyl-phosphate. Recent work with *E. coli* suggests that global, nonenzymatic, low-level lysine acetylation is also mediated by the reactive, high-energy metabolite acetyl-phosphate (AcP) and that this acetylation event is globally regulated by growth phase and metabolism (81). Weinert et al. compared the levels of protein acetylation from *E. coli* cells at different growth phases by using SILAC quantitative mass spectrometry (81). This analysis revealed that the bulk level of protein acetylation was dramatically increased in stationary phase. An increase in protein acetylation was also strongly correlated with an increase in AcP levels. Taken together, these results suggest that AcP may be directly involved in widespread, growth phase-dependent chemical (nonenzymatic) acetylation of *E. coli* proteins. The physiological significance of this phenomenon is not understood (81). Further support for the role of AcP as a nonspecific donor of acetyl groups in lysine acetylation in *E. coli* was recently reported (152). Direct acetylation by acetyl-CoA has also been suggested to be a mechanism of nonenzymatic acetylation in eukaryotes (163).

(ii) Validation of proteomic approaches. Because of its broad distribution in nature, RLA elicited a great deal of interest among biologists eager to define the role of RLA in cell physiology. However, several issues need to be addressed before we understand whether or not the function of specific proteins is under RLA control. Issues that need clarification are as follows.

(a) Nonenzymatic protein acetylation. Chemical, nonenzymatic lysine acetylation can occur when the pH is ≥8.0 (151, 164, 165). The autoacetylation activity of some proteins (166) further compounds this problem, leading to reporting of false-positive enzyme-driven acetylation. Therefore, multiple controls must be used to distinguish between autoacetylation and enzyme-driven acetylation, namely, the reaction substrates with the addition of inactive variants of the modifying acetyltransferase enzyme.

(b) Physiological relevance of multiple acetylation sites. Many of the reported acetylomes identify proteins with multiple acetylated lysine residues (~5 to 10 in some cases). This information contrasts sharply with observations for validated RLA targets such as acetyl-CoA synthetase (Acs) and other members of the acyl-CoA synthetases (discussed further below), in which the acetylation of a single lysine residue is necessary and sufficient to alter enzyme function (23, 28, 167). Recently, the first validated example of an Acs homologue from *Saccharopolyspora erythraea* that undergoes multiple acetylation events was reported, and the observations were validated *in vitro* and *in vivo* (168). However, of the four original acetylation events, only two were shown to have an effect on enzyme activity, highlighting the importance of *in vivo* and *in vitro* validation. It is imperative to determine the effect that each acetylated lysine may have on substrate binding, catalytic activity, or resistance to proteolytic activity to gain insights into the physiological importance of RLA.

(c) Possible overrepresentation of central metabolic enzymes. The fact that the majority of acetylated peptides identified are involved in central metabolism, specifically glycolysis, has been perhaps overemphasized. Notably, membrane-associated proteins, proteins present at low levels, or proteins not expressed under the culture conditions tested may represent important groups of targets under the control of RLA that are missed by the current methodology.

(d) Validation. Very limited and in some cases no validation of results obtained by mass spectrometry analyses has been reported in studies of bacterial acetylomes. Specifically, the function of the proteins identified as acetylation targets has not been analyzed *in vitro* to identify the modifying enzymes or whether or not acety-

TABLE 3 Validated substrates of prokaryotic lysine acetyltransferases

Acetyltransferase and protein substrate	Function	Reference(s)
Bacillus subtilis AcuA		
AcsA	AMP-forming acetyl-CoA synthetase	30
Escherichia coli Pka		
Acs	AMP-forming acetyl-CoA synthetase	177
RNase R	Stable RNA exoribonuclease	68, 92
RcsB	Response regulator for capsule synthesis	149
Mycobacterium smegmatis PatA		
MSMEG_4207	Universal stress protein	191
Acs	AMP-forming acetyl-CoA synthetase	178
FadD2, FadD4, FadD5, FadD10, FadD12, FadD13, FadD22, FadD35	AMP-forming acyl-CoA synthetases	192
Rhodopseudomonas palustris KatA		
Acs	AMP-forming acetyl-CoA synthetase	28
PrpE	AMP-forming propionyl-CoA synthetase	28
BadA, HbaA, AliA	AMP-forming aromatic and alicyclic acyl-CoA synthetases	28
Rhodopseudomonas palustris Pat		
Acs	AMP-forming acetyl-CoA synthetase	69
PrpE	AMP-forming propionyl-CoA synthetase	28
BadA, HbaA, AliA	AMP-forming aromatic and alicyclic acyl-CoA synthetases	69
PimA	AMP-forming pimeloyl-CoA synthetase	28
HcsA, FadD, FcsA, LcsA, IbuA	AMP-forming acyl-CoA synthetases that activate mono- and dicarboxylic acids	28
Salmonella enterica Pat		
Acs	AMP-forming acetyl-CoA synthetase	23, 169
PrpE	AMP-forming propionyl-CoA synthetase	56
Streptomyces lividans PatA		
AacS	AMP-forming acetoacetyl-CoA synthetase	31
Sulfolobus solfataricus Pat		
Alba	Chromatin protein	59

lation alters protein function or stability. At present, large-scale mass spectrometry results for bacterial acetylomes provide only putative targets until further validation is performed.

VALIDATED REVERSIBLE LYSINE ACYLATION TARGETS IN BACTERIA AND ARCHAEA

Discovery of RLA in Prokaryotes

The role of RLA in bacteria, specifically in relation to acetylation of metabolic enzymes, was discovered in *S. enterica*. See Table 3 for a to-date-comprehensive list of validated RLA targets in prokaryotes. The protein acetyltransferase of *S. enterica*, *Se*Pat, was first identified as the enzyme responsible for the acetylation and inactivation of Acs, the enzyme that activates acetate when present in the environment at low concentrations (≤10 mM) (24). Acs belongs to the acyl-CoA synthetase family (Pfam 00501), which convert acetate to acetyl-CoA (24). Acyl-CoA synthetases are ubiquitous across all domains of life (169–171), converting weak organic acids to their CoA thioesters through two half-reactions via an adenylated intermediate (172) (Fig. 10A):

$$\text{acetate} + \text{ATP} + \text{Acs} \rightarrow \text{acetyl-AMP} + \text{PP}_i + \text{Acs} \quad (1)$$

$$\text{acetyl-AMP} + \text{CoA} + \text{Acs} \rightarrow \text{acetyl-CoA} + \text{AMP} + \text{Acs} \quad (2)$$

As mentioned above, *Se*Acs activity is required during growth at low concentrations of acetate (≤10 mM). When the concentration of acetate in the environment is >10 mM, assimilation of acetate occurs via the phosphotransacetylase/acetate kinase (Pta/Ack) pathway (173).

*Se*Acs activity is regulated by the reversible acetylation of an active-site lysine residue, Lys609 (23). This discovery opened the doors to numerous studies on the regulation of metabolic enzymes by acetylation and shifted the focus from the regulation of histones by acetylation to acetylation as a means to regulate metabolism and physiology.

*Se*Acs activity was first predicted to be under the control of acetylation when it was observed that the sirtuin deacetylase (*Se*CobB) was needed under growth conditions in which acetate (10 mM) or propionate (30 mM) was the sole source of carbon and energy (23, 29). In support of this idea, the *Se*CobB protein was shown to deacetylate Acs *in vitro* (23), resulting in reactivation of the Acs enzyme. These findings revealed for the first time that acetylation was a means to modulate the activity of a metabolic enzyme (23). The modifying protein acetyltransferase of this bacterium, *Se*Pat, was subsequently discovered by selecting for derivatives of a Δ*cobB* strain that grew on 10 mM acetate (24). Deletion of the protein acetyltransferase (*Se*Pat) in a strain lacking *Se*CobB restored growth on both acetate (10 mM) and

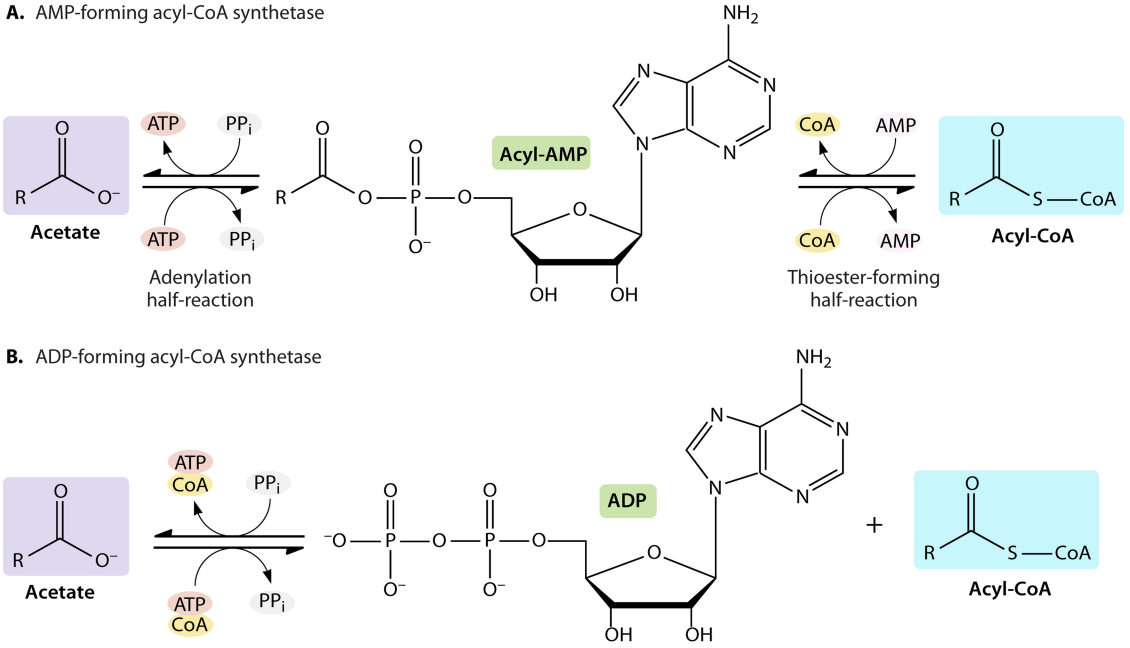

FIG 10 Synthesis of acyl-CoAs by AMP- and ADP-forming acyl-CoA synthetases. The AMP-forming and ADP-forming acyl-CoA synthetases convert organic acids to their CoA thioesters. AMP-forming acyl-CoA synthetases perform this reaction through two half-reactions via an adenylated intermediate (A), while the ADP-forming acyl-CoA synthetase reaction is driven by the energy of hydrolysis of the γ-phosphate of ATP (B).

propionate (30 mM). *In vitro* studies demonstrated that *Se*Pat could both acetylate *Se*Acs and propionylate the propionyl-CoA synthetase (PrpE) (24, 56).

The acetylated lysine residue (Lys609 in *Se*Acs) is required for adenylylation of organic acids and is universally conserved in the AMP-forming acyl-CoA synthetases. Acetylation of this lysyl residue prevents adenylylation of the acid substrate and blocks enzyme activity. Thus, *Se*Pat acetylation of the *Se*Acs active-site lysine blocks the conversion of acetate to acetyl-AMP (23), the first half-reaction catalyzed by *Se*Acs. It is proposed that the lysyl residue (i) aids in the orientation of the carboxylate moiety of the acid and phosphoryl groups of ATP for the in-line attack and (ii) stabilizes the transition state through positive-charge interactions (174, 175).

Acetylation of Acs is a conserved regulatory mechanism. Since its discovery, it has been shown that RLA controls Acs and other members of the acyl-CoA synthetases via acetylation and that this regulatory mechanism of metabolism is present in prokaryotes and eukaryotes (29, 176). Acetylation of Acs homologues by *Se*Pat homologues has also been demonstrated in *E. coli* (141, 177), *R. palustris* (69), *B. subtilis* (30), *Streptomyces* spp. (31), and *Mycobacterium* spp. (178). Insight into the need for posttranslational control of Acs activity was provided by studies that demonstrated that dysregulation of this enzyme caused a severe imbalance in the energy charge of the cell, leading to growth arrest (179). An expanded explanation of these effects is described below.

RLA in Gram-Negative Bacteria

Non-acyl-CoA synthetase targets of the *Salmonella enterica* protein acetyltransferase. It has been suggested that *Se*Pat can acetylate a variety of metabolic enzymes, including glyceraldehyde-3-phosphate dehydrogenase (GapA), isocitrate lyase (AceA), and the glyoxylate shunt regulator isocitrate dehydrogenase kinase/phosphatase (AceK) (153). However, difficulties in reproducing these results by others have been reported (28). These discrepancies need to be clarified before any conclusions about the involvement of RLA in the modulation of such key enzymes can be validated.

In *Escherichia coli*, the protein acetyltransferase (Pka) alters the fate of RNase R. Results from early studies suggested that the Pat homologue from *E. coli*, *Ec*Pka, acetylated the RNA polymerase alpha subunit; however, results from subsequent analyses failed to confirm this claim (180, 181). A bona fide substrate of *Ec*Pka is the exoribonuclease RNase R, shown to degrade highly structured mRNA. RNase R is acetylated during exponential phase, destabilizing the protein and making it prone to proteolytic degradation (68, 92). This is the first example of acetylation by a *Se*Pat homologue affecting protein stability and thus enzyme activity. Notably, RNase R cannot be deacetylated by the *E. coli* sirtuin CobB, the only known protein deacetylase in this bacterium, and therefore is not a reversible modification (68).

In *Rhodopseudomonas palustris*, RLA controls the activity of many AMP-forming acyl-CoA synthetases. The role of RLA in the physiology of the purple nonsulfur photosynthetic alphaproteobacterium *R. palustris* has been investigated (28). In this bacterium, RLA modulates the activity of enzymes involved in the anaerobic catabolism of aromatic organic acids and other fatty acids. Results from proteomics global analyses indicate that *Rp*Pat acetylates many acyl-CoA synthetases (AMP forming) in addition to Acs and propionyl-CoA synthetase (PrpE), including those involved in activating short-, medium-, and long-chain fatty acids and aromatic acids (e.g., BadA, HbaA, AliA, PimA, HcsA, FadD, FcsA, LcsA, and IbuA) (28). It is significant that all of the verified *Rp*Pat substrates from this study were acyl-CoA synthetases. These data strongly suggest that *Rp*Pat may specifically recognize and regulate this class of enzymes via a negative-feedback mechanism.

When an *Rp*Pat substrate, benzoyl-CoA synthetase (BadA), was acetylated *in vivo* in the absence of *Rp*Pat, Crosby et al. predicted the existence of a second *R. palustris* protein acetyltransferase (69). A single-domain GNAT, *Rp*KatA (for K [Lys] acetyltransferase A), was identified based on its limited sequence homology (33% identical over 64 residues within the GNAT domain) to other known protein acetyltransferases. *Rp*KatA also acetylated the conserved catalytic lysine of acyl-CoA synthetases whose substrates included short-, medium-, long-, and branched-chain fatty acids in addition to aromatic organic acids (28, 69). It is noteworthy that although *Rp*KatA has enzymatic capabilities similar to those of *Rp*Pat (type I GNAT), it is a much smaller protein, comprised of only a catalytic domain (type IV GNAT).

Insights into the role of *Rp*KatA were obtained by performing mass spectrometry-based proteomics analysis. Briefly, four acyl-CoA synthetases (BadA, AliA, HbaA, and PrpE) were acetylated in a *pat* deletion strain, but no acetylation of these proteins was seen in a *pat katA* double mutant (28). From *in vitro* studies, the authors learned that *Rp*Pat and *Rp*KatA had different substrate specificities for the acyl-CoA synthetases of *R. palustris*. For example, there are two acyl-CoA synthetases, hexanoyl-CoA synthetase A (HcsA) and long-chain acyl-CoA synthetase A (LcsA), which were acetylated by *Rp*Pat but were not acetylated by *Rp*KatA (28). It is not known how *Rp*KatA and *Rp*Pat recognize their substrates or why there is overlapping activity between the enzymes.

In addition to having two protein acetyltransferases, *R. palustris* also encodes two protein deacetylases, a sirtuin deacetylase (SrtN) and a Zn(II)-dependent lysine deacetylase (LdaA). Genetic evidence suggests that both deacetylases play a role in regulating acyl-CoA synthetases in *R. palustris* (28).

RLA in Gram-Positive Bacteria

RLA controls acetyl-CoA synthetase activity in *Bacillus subtilis*. In *B. subtilis*, the GNAT *Bs*AcuA (type IV protein acetyltransferase) is comprised of only a single GNAT domain and has no significant sequence homology to *Se*Pat (type I protein acetyltransferase). However, a structure of a *Bs*AcuA homologue resolved from *Exiguobacterium sibiricum* demonstrated that *Bs*AcuA contains the conserved GNAT domain (PDB accession number 2Q04) (78). *Bs*AcuA acetylates and inactivates acetyl-CoA synthetase A (AcsA) at the conserved catalytic lysine (Lys549) (30). Deacetylation of $Bs\text{AcsA}^{Ac}$ (acetylated *Bs*AcsA) can occur by either of the two protein deacetylases of *B. subtilis*, the Zn(II)-dependent *Bs*AcuC deacetylase and/or the sirtuin *Bs*SrtN (30, 182).

The genes encoding *Bs*AcuA (GNAT) and *Bs*AcuC (deacetylase) are located within the *acuABC* operon (183) and are divergently transcribed from *acsA* (target). It was initially thought that the *acuABC* operon was involved in acetoin utilization, as deletion of *acuA* caused a growth defect when cells were grown on acetoin (183). However, it has since been shown that the acetoin utilization pathway is encoded by *acoABCLR* in *B. subtilis* (184). At present, it is unclear if or how *Bs*AcuB is involved in the *Bs*AcuA- and *Bs*AcuC-dependent regulation of *Bs*AcsA or if acetoin utilization is either directly or indirectly regulated by acetylation.

Acs from *Streptomyces lividans* is the exception to the Acs acetylation paradigm. Metabolic regulation in actinomycetes like *Streptomyces* is of interest because of the diverse natural products that they produce (185–188). *S. lividans* encodes a protein acetyltransferase, *Sl*PatA (type II), which also acetylates AMP-forming acyl-CoA synthetases, including *S. lividans* acetoacetyl-CoA synthetase (*Sl*AacS) and *S. enterica* Acs (31). *Sl*PatA only weakly modified the *S. lividans* Acs homologue. However, it efficiently acetylated the related enzyme *Sl*AacS both *in vitro* and *in vivo* (31). AacS is present in all domains of life, and this work provided the first example of the regulation of its activity by acetylation. Recently, the structure of *Sl*AacS was reported, which provided for the first time an ordered view of the 30-residue extension of the C terminus of this type of enzyme, and it was suggested that such an extension may interact with catalytic residues of the N-terminal domain (189). A comparison of *Sl*Acs and *Sl*AacS would provide valuable insights into the determinants that make AMP-forming acyl-CoA synthetases good substrates for the *Sl*PatA enzyme.

*Sl*PatA is the first characterized Pat homologue that does not efficiently acetylate its cognate Acs enzyme *in vitro* (31), suggesting that *Sl*PatA may not be the enzyme responsible for Acs acetylation in *S. lividans*. Alternatively, acetylation of *Sl*Acs by *Sl*PatA may require additional factors that are not required by *Se*Pat and *Rp*Pat for acetylation of Acs orthologues from those organisms, or *Sl*Acs simply is not enzymatically acetylated.

S. lividans encodes two sirtuin deacetylases, CobB1 and CobB2, and a Zn(II)-dependent AcuC-type deacetylase. Work with the closely related organism *Streptomyces coelicolor* demonstrated that Acs was acetylated and that CobB1 deacetylated Acs *in vitro* (190). However, the acetyltransferase responsible for the acetylation of *S. coelicolor* Acs was not identified.

Recently, studies showed that in another actinomycete, *Saccharopolyspora erythraea*, a homologue of the GNAT-related AcuA enzyme from *B. subtilis* acetylates the *S. erythraea* Acs enzyme at four different positions and that in a mutant of *S. erythraea* lacking the *Sa*AcuA enzyme, *Sa*Acs is not acetylated at all (168). The *Sa*AcuA enzyme is the first of its class to be experimentally shown to target several residues of an Acs homologue, as described above. Whether AcuA homologues in other actinomycetes are responsible for the single or multiple acetylation of Acs in this class of microorganisms remains to be determined.

In *Mycobacterium* spp., the universal stress protein is under RLA control. *M. tuberculosis* and *M. smegmatis* encode unique protein lysine acetyltransferases (*Mt*PatA and *Ms*PatA, respectively). In these organisms, the GNAT domain is attached to a cyclic AMP (cAMP) binding domain (type III) (178, 191). cAMP allosterically activates *Mt*PatA and *Ms*PatA, enhancing their activity >2-fold (178, 191–194).

*Ms*PatA acetylates a universal stress protein (USP) (MSMEG_4207) at a single lysine residue, and acetylation increases in the presence of cAMP (191). The *in vivo* significance of USP acetylation was not tested, likely because the function of most USPs is unclear, but there is evidence suggesting that USPs provide resistance to various stressors (reviewed in reference 195).

*Ms*PatA and *Mt*PatA share 57% identity; thus, they may have similar substrates, except for USP, which is not conserved in mycobacteria that encode homologues of *Ms*PatA. Based on these findings, Xu et al. used *Ms*PatA to acetylate whole-cell lysates with or without the acetyl-CoA analogue chloroacetyl-CoA. These authors identified *Ms*Acs as an acetylated protein target of *Ms*PatA (178). Because *Ms*Acs could not be overproduced in *E. coli*, *in vitro* experiments aimed at showing acetylation of *Ms*Acs by *Ms*PatA were not performed (178). Using an alternative approach, these authors purified *Mt*Acs (76% identical to *Ms*Acs) and demonstrated *Ms*PatA-dependent acetylation of *Mt*Acs at the expected

catalytic lysine residue, which abolished *Mt*Acs activity (178). Although the degree of identity between *Mt*PatA and *Ms*PatA is high, there is precedent in the literature of Acs enzymes that are very poorly acetylated by Pat enzymes (e.g., *Sl*Acs). Therefore, in the absence of experimental evidence, it is premature to conclude that *Ms*Acs is a substrate of *Ms*PatA.

An independent study identified eight additional acyl-CoA synthetases as the substrates of *Ms*PatA (192). The single protein deacetylase in *M. tuberculosis*, an NAD^+-dependent sirtuin homologue (MRA_1161, from H37Ra), deacetylated *Mt*Acs and all eight acyl-CoA synthetases *in vitro* (178, 192), suggesting that that this likely constitutes a regulatory system comparable to the RLA systems found in *S. enterica* and *R. palustris* (196). The authors were able to demonstrate that acetylation in *M. tuberculosis* was dependent upon intracellular cAMP levels by examining the acetylation level of known targets under conditions of various cAMP concentrations. Acetylation of the acyl-CoA synthetase targets was seen only under conditions of higher levels of cAMP (178, 192).

RLA in Archaea

The first studies examining protein acetylation and deacetylation in archaea were performed with *Halobacterium salinarum* (previously *H. halobium*), in which a 2Fe-2S ferredoxin protein was identified to be acetylated at a specific lysine residue (Lys118) (197). While this was the first example of protein acetylation in archaea, no further studies of acetylated proteins involved in processes other than gene expression have been reported.

Acetylation of the *Sulfolobus solfataricus* chromatin protein (Alba). As mentioned above, little is known about acetylation in archaea, even though many archaeal species have acetyltransferase and deacetylase homologues. Some archaea encode histone proteins similar to those of eukaryotes. However, the archaeal histones differ in that they do not contain the flexible N-terminal tails and are not posttranslationally modified (198, 199). In addition to histones, archaea have another chromatin protein known as Sso10b or Alba (for acetylation lowers binding affinity). When bound to DNA, Alba inhibits transcription (200, 201).

Investigators discovered that Alba homologues purified from *Sulfolobus* spp. were 84-Da larger than predicted. Because an acetyl group adds 42 Da to the protein mass, it was suggested that the Alba proteins were acetylated at two sites (200, 202). A type IV GNAT family acetyltransferase, *Ss*Pat (single GNAT domain), was identified by homology to *Se*Pat and was shown to acetylate Alba *in vitro* at Lys16 (59). A subsequent study showed that acetylation of Lys16 decreased the ability of Alba to bind DNA by ~3-fold (59). However, those authors determined that Alba is a relatively poor substrate for *Ss*Pat and that other substrates may exist in *S. solfataricus* (74). Lys16 of Alba was shown to be deacetylated by the sirtuin deacetylase in *S. solfataricus* (Sir2) (200), which increased the affinity of Alba for DNA (200). The control of Alba by acetylation and deacetylation seems to mirror histone regulation in eukaryotes, providing an example of a conserved regulatory process.

RLA Targets Whose Modifying Acetyltransferases Are Not Known

A large number of proteins have been reported to be acetylated, but the identity of the modifying GNAT has not been discovered. Two of these examples include the transcriptional regulator RcsB and the chemotaxis response regulator CheY.

In addition to Acs, an *E. coli* proteome array incubated with *Se*Pat (92% identical to *Ec*Pka) and radiolabeled [1-^{14}C]acetyl-CoA suggested that *Se*Pat acetylated several proteins, including the bacterial transcription factor RcsB (149). RcsB is involved in regulating the expression of genes that affect flagellar and capsule synthesis as well as cell division (203, 204). Acetylation of RcsB decreases its ability to bind DNA, an effect that is reversed by incubation of $RcsB^{Ac}$ with the sirtuin *Ec*CobB and NAD^+ (149). More recently reported work did not find direct evidence that *Ec*Pka acetylates RcsB in *E. coli*, leaving the identity of the acetyltransferase that modifies RcsB in these bacteria unknown (205).

The response regulator CheY, involved in bacterial chemotaxis, is predicted to be under the control of RLA (166, 206). While the phosphorylation of CheY has been extensively studied, the effect of acetylation on the activity of the protein is still poorly understood (207–209). Acetylation of CheY inhibits binding to three of its interacting partners, the CheA kinase, the CheZ phosphatase, and the flagellar motor switch FliM protein (166). CheY is acetylated at multiple sites *in vivo*, and the majority of the acetylated residues are grouped on the surface of the protein near the C terminus, the region that binds to the protein targets (210).

While there is *in vivo* and *in vitro* evidence that the CobB sirtuin deacetylates CheY, the identity of the acetyltransferase is unknown (209). Previously, acetylation of CheY was hypothesized to occur via either autoacetylation or acetyl-CoA synthetase-dependent acetylation by some unknown mechanism (206, 211). To date, studies of CheY have been performed by chemical acetylation of the protein using acetic anhydride (208). However, this method does not acetylate CheY to the same extent as what is seen for the protein *in vivo* (166, 206). It is currently hypothesized that there must be a GNAT responsible for the acetylation of CheY and that it is yet to be discovered.

GNAT STRUCTURE AND SUBSTRATE SPECIFICITY

Structural divergence of ADP-forming acyl-CoA synthetases. As mentioned above, the large domain of type I and type II GNATs is homologous to ADP-forming acyl-CoA synthetases. This catalytic mechanism involves the transfer of a phosphate group to a conserved histidinyl residue located within a flexible loop in subdomain 2 (212) (Fig. 10B). *Ec*Pka and *Se*Pat have divergent sequences in the flexible loop region and lack the catalytic residue, suggesting that their ADP-forming acyl-CoA synthetase domains may lack enzymatic activity. Other protein acetyltransferases, like *Rp*Pat, encode the catalytic histidine, but whether or not *Rp*Pat maintains acyl-CoA synthetase activity has not yet been investigated.

***Mycobacterium tuberculosis* PatA is a sensor of carbon quality.** The crystal structure of *Mt*PatA has been resolved in the presence and absence of cAMP and revealed an intricate regulatory mechanism (PDB accession number 4AVB) (194) (Fig. 11). Structural studies demonstrated that *Mt*PatA can exist in either an active or an autoinhibited state (194). In the absence of cAMP, *Mt*PatA adopts the autoinhibited state, in which the C-terminal helix (lid) blocks the entrance of the protein substrate into the active site of the GNAT domain (PDB accession number 4AVA). In the active state, the cAMP binding domain is rotated 40° relative to the GNAT domain, causing the inhibitory lid to refold and swing away, exposing the active-site cleft (194) (Fig. 11). Binding of cAMP stabilizes the active state of *Mt*PatA, enabling acetylation of the target. Acetyl-CoA copurified with *Mt*PatA and was present

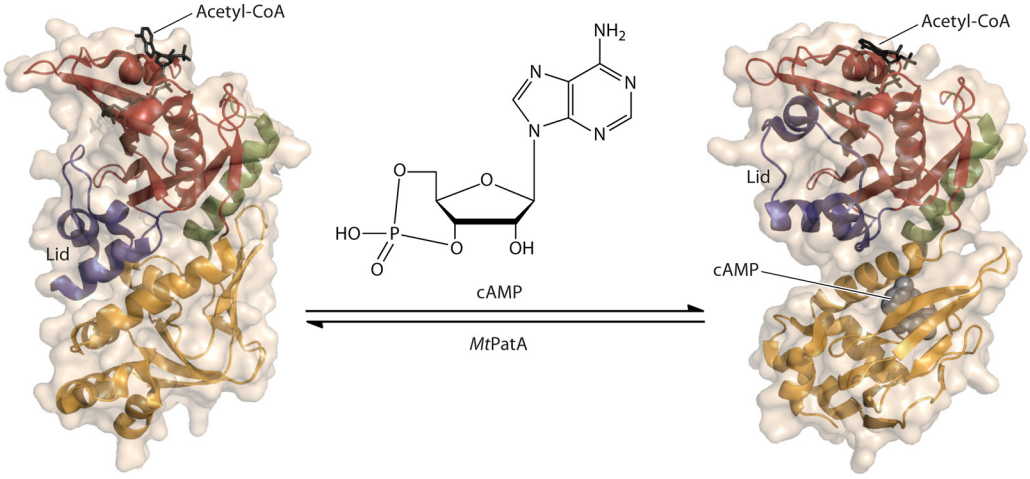

FIG 11 Binding of cAMP induces a 40-Å structural change in *M. tuberculosis* PatA. In the absence of cAMP, *Mt*PatA adopts an autoinhibited state, where a "lid" (blue) blocks the entrance of the substrate to the active site of the GNAT domain (red). In the presence of cAMP, the cAMP binding domain (gold) rotates 40° relative to the GNAT domain. This causes the lid to swing away from the GNAT domain, exposing the active-site cleft. Also shown are acetyl-CoA (black sticks), cAMP (gray spheres), and the C-terminal helix (green). The PDB accession numbers are 4AVA for the *Mt*PatA structure and 4AVB for the *Mt*PatA structure with cAMP.

in both crystal structures, demonstrating that it binds tightly to the enzyme and that *Mt*PatA is poised to respond to cAMP levels (194).

Determinants Needed for Recognition and Acetylation of Protein Targets by GNATs

The ternary structure of the *Tetrahymena thermophila* GNAT, *Tt*Gcn5, in complex with an 11-residue peptide from histone H3 and CoA (PDB accession number 1QSN) (Fig. 12A) revealed a constellation of interactions between GNATs and their protein substrates (49) (Fig. 12B). Notably, CoA binding to *Tt*Gcn5 triggers structural changes that facilitate its interactions with the protein substrate. From the above-mentioned structure, one can see that the role of the catalytic residue (Glu122) is to abstract a proton from the ε-amino group of Lys14 via an ordered water molecule bridging the two residues. Further positioning of Lys14 is afforded by hydrophobic interactions between residues in *Tt*Gcn5 and methylene groups and the ε-amino of the lysyl side chain. The glycyl side chain next to Lys14 interacts with *Tt*Gcn5 through van der Waals forces, most likely introducing flexibility into the substrate protein. Binding of the protein substrate to *Tt*Gcn5 positions the G-**K**-X-P motif (**K** referring to the acetylation target) of the former in close proximity to acetyl-CoA in the active site of *Tt*Gcn5. Such a position is maintained via interactions between the prolyl side chain and CoA.

Structure of a GNAT-protein substrate complex. Recently, the first structure of a GNAT family member in complex with a protein substrate with a tertiary structure was reported (71, 213). The GNAT domain from *S. lividans Sl*PatA (*Sl*PatA^{GNAT}) was crystallized in complex with the C-terminal domain of *S. enterica* Acs (*Se*Acs^{CTD}) (71) (Fig. 13A). A comparison of the *Sl*PatA^{GNAT}-*Se*Acs^{CTD} and *Tt*Gcn5-peptide structures revealed that (i) a glycine residue (G608) preceding the target lysine (K609) was important for positioning the lysine for interaction with *Sl*PatA^{GNAT} and (ii) a hydrophobic pocket in *Sl*PatA^{GNAT} positioned the lysine side chain (K609) near the catalytic glutamate (E123) of the GNAT (Fig. 13B). The interaction surface of the *Sl*PatA^{GNAT}-*Se*Acs^{CTD} acetylation complex was more extensive than the surface observed for *Tt*Gcn5-peptide interactions, indicating that *Sl*PatA recognizes substrate sequences outside the flexible loop containing the target lysine (K609). These interactions included

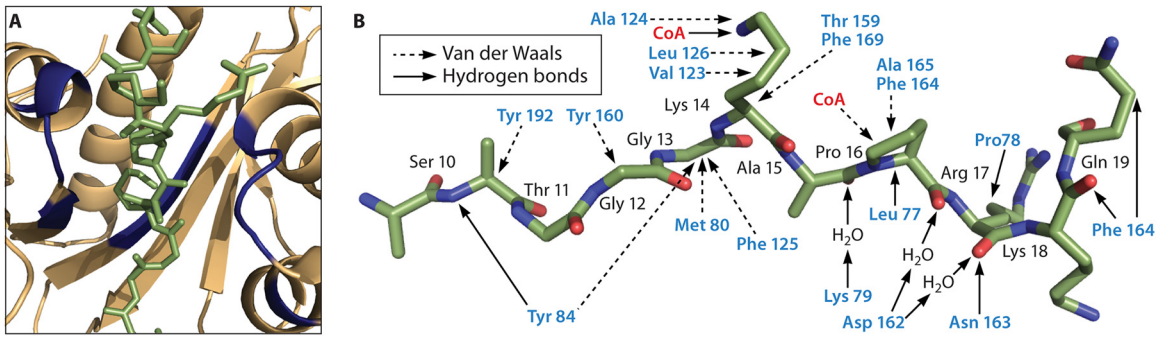

FIG 12 Interactions between the *T. thermophila* Gcn5 protein and a peptide substrate. (A) The structure of *Tt*Gcn5 and a histone H3 11-peptide residue (PDB accession number 1QSN) demonstrates that the presence of CoA (not shown) causes structural changes that may facilitate interactions with its protein substrate (interacting residues are shown in blue). (B) Molecular interactions of *Tt*Gcn5 (blue) with the peptide substrate (green).

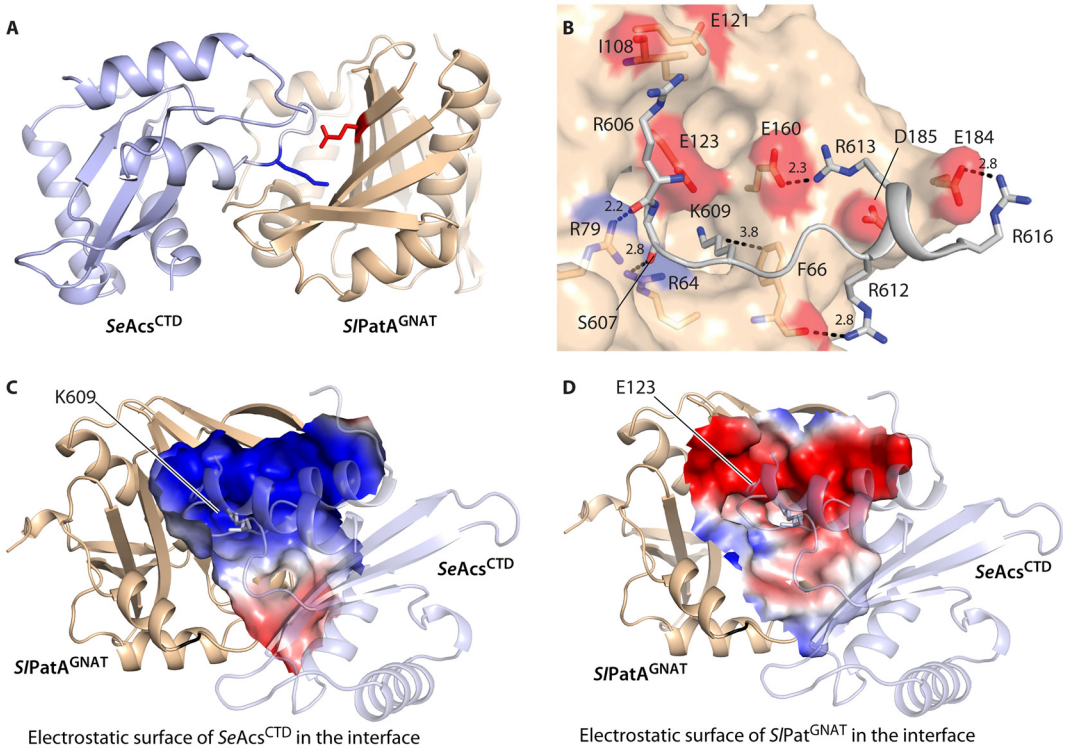

FIG 13 Molecular interactions of *S. lividans* PatA^{GNAT} and *S. enterica* Acs^{CTD}. (A) Crystal structure of the interactions between *S. lividans* PatA^{GNAT} and *S. enterica* Acs^{CTD} (PDB accession number 4U5Y). The *Sl*PatA^{GNAT} catalytic residue (E123) is shown in red sticks, and the acetylated lysine of *Se*Acs^{CTD} (K609) is shown in blue sticks. (B) Interactions between *Sl*PatA^{GNAT} (surface) and *Se*Acs^{CTD} (sticks). (C and D) Electrostatic potential of the *Sl*PatA^{GNAT}-*Se*Acs^{CTD} surface interface, with negatively charged regions in red, positively charged regions in blue, and neutral residues in white. (Adapted from reference 71. © American Society for Biochemistry and Molecular Biology.)

complementary ionic interactions of positively charged side chains in *Se*Acs^{CTD} with negatively charged side chains in *Sl*PatA^{GNAT} (Fig. 13C and D). Reversing the charges in either *Se*Acs^{CTD} or *Sl*PatA^{GNAT} significantly decreased interactions between these proteins (71).

Diversity of determinants in the motif containing the acetylation site. All bona fide substrates of *R. palustris Rp*Pat are AMP-forming acyl-CoA synthetases and display a high degree of conservation surrounding the acetylation site. A consensus sequence at the site of acetylation can be approximated by the motif PX$_4$G**K** (23, 169) (Fig. 14A). The Gly residue preceding the target lysine is conserved in *Rp*Pat substrates, as seen with the *Tt*Gcn5 substrate mentioned above, and may be a common feature of GNAT substrates (49). However, recent results support the conclusion that this motif is not sufficient for acetylation to occur. *Rp*MatB, an acyl-CoA synthetase which activates the dicarboxylic acid methylmalonate to methylmalonyl-CoA (214), contains the PX$_4$G**K** motif but is not acetylated by *Rp*Pat (215) (Fig. 14B).

The term "acetylation motif" is misleading because it oversimplifies what is encoded in this motif. While it is true that the PX$_4$G**K** motif identified for AMP-forming acyl-CoA synthetase acetylation targets is necessary for acetylation to occur, it is not sufficient. Alanine scanning of 14 residues surrounding the acetylation site of *Rp*PimA (a bona fide substrate of *Rp*Pat) demonstrated that nearly half of them were important for acetylation by *Rp*Pat, whereas only two were required for *Rp*PimA enzymatic activity (23, 28, 167). Interestingly, one acyl-CoA synthetase was identified in *R. palustris*, which appeared to evade acetylation through the presence of a leucine residue two positions upstream of the conserved lysine. Changing of the leucine to a valine residue restored recognition of the substrate and its acetylation. Collectively, these results emphasize the fact that the presence of the acetylation motif in AMP-forming acyl-CoA synthetases is not a good predictor of a protein being under RLA control.

Use of Protein Chimeras To Probe GNAT Substrate Specificities

To investigate *Rp*Pat specificity, a series of chimeric proteins were constructed, in which portions of *Rp*MatB (methylmalonyl-CoA synthetase; not acetylatable) were replaced with the corresponding sequences from known *Rp*Pat substrates (Fig. 14C). A series of chimeric proteins were constructed between *Rp*Pat and pimelate-CoA synthetase (*Rp*PimA) or benzoate-CoA synthetase from *Burkholderia xenovorans* (*Bx*BclM) (28, 216). The introduction of residues from *Rp*PimA or *Bx*BclM into *Rp*MatB allowed the *Rp*PimA-*Rp*MatB and *Bx*BclM-*Rp*MatB chimeras to be recognized and acetylated by *Rp*Pat. Significantly, *Rp*PimA-*Rp*MatB chimeras with *Rp*PimA residues located ~20 Å away from the target lysine (K488) allowed *Rp*Pat to recognize and acetylate *Rp*MatB. These data indicated that *Rp*Pat recognizes additional structural elements in protein substrates in addition to the residues immediately surrounding the target lysine, as seen with *Sl*PatA. This information may help account for the substrate spec-

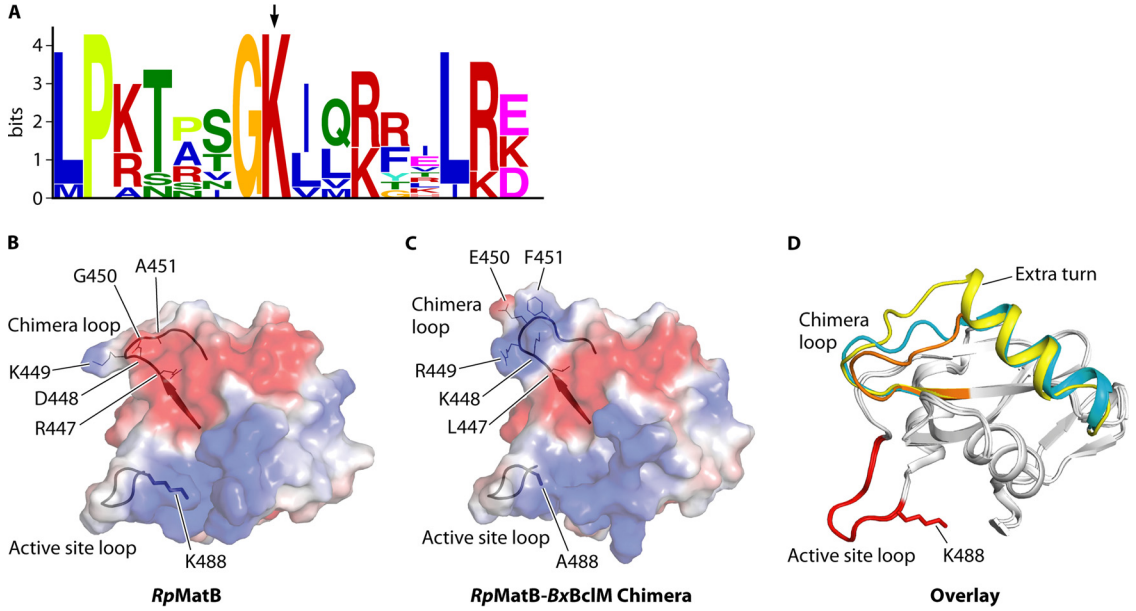

FIG 14 Acetylation determinants outside the motif containing the acetylation site. (A) Consensus motif containing the acetylation site (indicated by the arrow) generated from the alignments of acyl-CoA synthetases acetylated by *R. palustris* Pat (*Rp*Pat). The letter height corresponds to the frequency of a particular amino acid residue in that position. (B) Electrostatic potential of *Rp*MatB (methylmalonyl CoA synthetase), a protein that is not acetylated by *Rp*Pat. Negatively charged regions are shown in red, and positively charged regions are shown in blue. (C) Electrostatic potential of the *Rp*MatB and *B. xenovorans* BclM (benzoate:CoA synthetase) chimera protein (*Rp*MatB-*Bx*BclM chimera B3), a protein that is acetylated by *Rp*Pat. Negatively charged regions are in red, and positively charged regions are in blue. (D) Overlay of the C-terminal domain of the *Rp*MatB-*Bx*BclM chimeras (PDB accession numbers 4GXQ for B1 and 4GXR for B3) aligned with the C-terminal domains of *Rp*MatB (PDB accession number 4FUQ), with the *Bx*BclM-derived residues of the B1 chimera in yellow, *Bx*BclM-derived residues of the B3 chimera in orange, and wild-type *Rp*MatB residues in cyan. The consensus motif containing the acetylation site ($PX_4G\mathbf{K}$) is shown in red in the active-site loop, with the acetylated lysine residue (K488) shown as red sticks. (Adapted from references 28 [panel A] and 215 [panels B to D]. © American Society for Biochemistry and Molecular Biology.)

ificity of protein acetyltransferases for their structurally diverse substrates.

The three-dimensional crystal structure of the *Bx*BclM-*Rp*MatB chimera identified a loop (named chimera loop) that is important for recognition by *Rp*Pat (215) (Fig. 14D). It seems that the shape and electrostatic potential of the chimera loop play important roles, as minor changes in the loop allow an acyl-CoA synthetase to "escape" acetylation by *Rp*Pat (214). This indicates that although acetylation motifs may suggest that a protein is controlled by RLA, each substrate should be validated experimentally, as structural elements outside the motif can affect the ability of the acetyltransferase to recognize and acetylate the target.

ROLE OF RLA IN MAINTAINING METABOLIC HOMEOSTASIS

Acetyl-CoA, Energy Charge, NAD^+, and cAMP Link RLA to Central Metabolism

Both components of the RLA system (acylation and deacylation) involve the essential coenzymes CoA and NAD^+. Acylation requires acyl-CoA thioesters, connecting this process to CoA homeostasis, carbon load, and energy charge, while the sirtuin-catalyzed deacylation reaction requires NAD^+, an indicator of high energy levels in the cell. Acylation is further regulated by metabolic cofactors in acetyltransferases such as the *Mycobacterium* Pat proteins, which respond to cAMP levels, an indicator of the quality of the carbon source available in the cell. As a result, RLA modifies proteins in response to the metabolic state of the cell, specifically reflecting the cellular energy and carbon status.

Acetylation and acetyl-CoA levels. Bacterial protein acetyltransferases use acetyl-CoA as a substrate, linking protein acetylation to acetyl-CoA levels, which are regulated by the metabolic activity of the cell. The two-domain Pat homologues *Se*Pat, *Ec*Pka, *Rp*Pat, and *Sl*PatA have large regulatory domains that bind acetyl-CoA to allosterically regulate acetyltransferase activity (72). Acetyl-CoA is a metabolite linked to many pathways, including carbon utilization (e.g., glycolysis), the tricarboxylic acid (TCA) cycle, the acetate kinase/phosphotransacetylase pathway, and fatty acid biosynthesis/degradation (Fig. 15).

Although some insights into the reasons why AMP-forming acyl-CoA synthetases are regulated by RLA have been reported (see below), there may be a number of other reasons why cells control this class of enzymes so carefully. Not all acyl-CoA synthetases are regulated by acetylation. In prokaryotes, data have been reported about the propionylation of propionyl-CoA synthetase (PrpE), a modification that affects the same lysine as acetylation does, also abolishing the activity of the enzyme (56). The use of RLA to control acyl-CoA synthetases by acetylation or propionylation is not unique to *Gammaproteobacteria*, since there is abundant evidence of the same type of control happening in the alphaproteobacterium *R. palustris*. The common theme here is that RLA helps maintain a balance in the intracellular acetyl-CoA (or propionyl-CoA) pools, while an acyl-CoA synthetase (MatB) that contributes to the succinyl-CoA pool is not under RLA control (28, 214). It is possible that acetylation of these AMP-forming acyl-CoA synthetases may control CoA homeostasis by preventing the depletion of CoA or buildup of acetyl-CoA or propionyl-CoA.

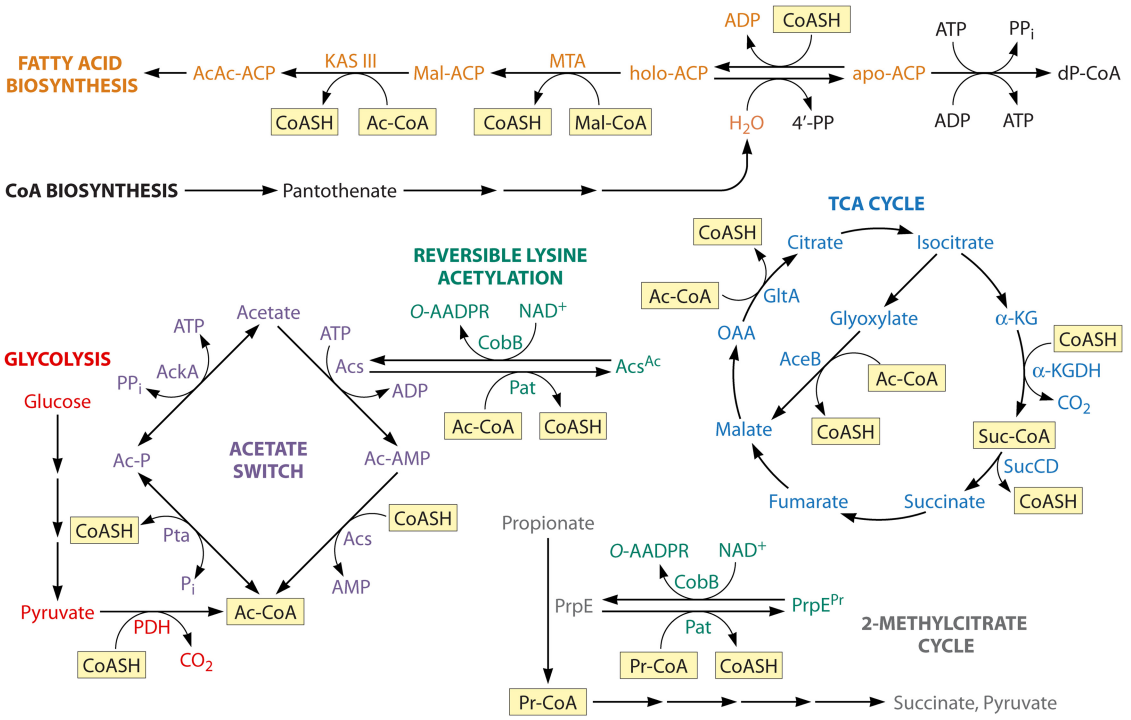

FIG 15 CoA homeostasis. Shown is a schematic of the contributions of CoA and acetyl-CoA to cellular metabolism. CoASH, coenzyme A; Ac-CoA, acetyl-CoA, *O*-AADPR, *O*-acetyl-ADP-ribose; Ac-P, acetyl-phosphate; Ac-AMP, acetyl-AMP, PP_i, pyrophosphate; Pr-CoA, propionyl-CoA; α-KG, alpha-ketoglutarate; OAA, oxaloacetate; Suc-CoA, succinyl-CoA; dP-CoA, dephospho-coenzyme A; AcAc-ACP, acetoacetyl-acyl carrier protein; KAS III, β-ketoacyl-(acyl-carrier-protein) synthase III; MTA, malonyl-CoA:acyl carrier protein transacylase; PDH, pyruvate dehydrogenase complex; α-KGDH, α-ketoglutarate dehydrogenase complex; SucCD, succinyl-CoA synthetase.

Possible effects on CoA homeostasis. CoA is an essential metabolic cofactor, and CoA homeostasis is important for cell survival. As an acyl carrier group, CoA activates the carbonyl groups of carboxylic acids, including fatty acids and amino acids. The resulting thioester bond increases the electrophilicity of the carbonyl carbon, facilitating nucleophilic attacks and thus making the carbonyl carbon more prone to react with thiolates, hydroxyl, and amino groups (217, 218). Reactive acyl-CoA thioesters are used by ~4% of all known enzymes, which catalyze >100 reactions involved in diverse cellular processes, including the TCA cycle, fatty acid degradation, and fatty acid, amino acid, and secondary-metabolite biosynthesis (219, 220) (Fig. 15). Due to both limited substrate availability and allosteric regulation of central metabolic enzymes, CoASH and acyl-CoA control metabolic flux through glycolysis and the TCA cycle (221–227).

CoASH, acetyl-CoA, succinyl-CoA, and malonyl-CoA comprise the bulk of the CoA pool (228). The availability of nutrients, phase of growth, and environmental conditions all affect the CoA pool and can cause the balance of acyl-CoA species to be altered by more than an order of magnitude in a matter of minutes (220, 229–231). For example, acetyl-CoA is the major species during exponential growth on glucose (300 μM), while CoASH is the predominant species during growth on acetate (100 μM) (228).

Because CoASH and acyl-CoA availability affects many cellular processes, an imbalance in CoA homeostasis results in profound consequences for cellular metabolism. For example, depletion of CoA stalls protein synthesis and reduces the supply of acyl carrier protein (ACP). Protein synthesis is stalled by depletion of CoA due to the lack of available acetyl-CoA as well as inhibition of the TCA cycle and production of amino acid precursors (232). Reduced levels of ACP limit fatty acid biosynthesis, ultimately resulting in reduced phospholipid synthesis (232–234). Due to the deleterious effects on the cell caused by an imbalance in CoA homeostasis, there must be tight control over both the total CoA pool as well as the relative concentrations of the various CoA species, in response to the metabolic status of the cell. Given that the RLA system recycles acylated CoAs, this could be a mechanism for the maintenance of CoA homeostasis.

Effect of RLA on energy charge. Insights into why acyl-CoA synthetases are under RLA control have been reported for *S. enterica* (179). From this work, these authors learned that in this bacterium, an imbalance in the protein acetyltransferase (*Se*Pat)/sirtuin deacetylase (*Se*CobB) ratio has a profound effect on cell growth under conditions that depend on the activity of acetyl-CoA synthetase (*Se*Acs), i.e., ≤10 mM acetate as the sole source of carbon and energy. Results from experiments where *Se*Pat was ectopically synthesized under the control of an inducible promoter showed that incremental levels of *Se*Pat eventually arrested growth because the energy charge of the cell was lowered to a level (0.17) that could not support growth. The depletion of ATP and the concomitant production of AMP were determined to be the reasons for the drop in the energy charge; that is, the cell did not have enough ATP to convert AMP to ADP so the ATPase could synthesize more ATP, thus restoring the energy charge of the cell.

Deacetylation and NAD^+ levels. Sirtuins require NAD^+ as a cosubstrate, linking deacetylation to the availability of NAD^+ in the cell. NAD^+ is the oxidized form of NADH, an important electron donor to the electron transport system, which generates the

proton motive force that drives the synthesis of ATP by the membrane-bound ATPase. When bacteria are grown under conditions with differing nutritional and oxygen availability, the NAD^+ pools have greater variation than do the NADH pools (130). This suggests that the NAD^+ pool is (i) dynamic and (ii) an important reporter of the cellular carbon and energy status. For example, growth conditions that generate high NAD^+ levels, like aerobic respiration, could cause an increase in the deacetylation of sirtuin targets. A recent report provided evidence of a new and unprecedented role for NAD^+ in cell physiology. Cahová et al. presented experimental evidence supporting the idea that bacteria stabilize RNA molecules by capping their 5′ end with NAD^+ (235). Whether NAD^+-capped RNAs are substrates for sirtuins is an intriguing possibility that should be explored.

Regulation of protein acetylation by cAMP. In *E. coli*, cAMP is involved in catabolite repression, a process in which cells preferentially use glucose and utilize other available carbon sources only once glucose has been depleted. The global transcriptional regulator Crp mediates this cAMP-dependent response (236). In mycobacteria, cAMP not only plays a role in basic physiology but also acts as a secondary messenger and is involved in the rerouting of host signaling during infection (237). Notably, *M. tuberculosis* has 15 adenylate cyclase enzymes for the production of cAMP, whereas *E. coli* has only 1 (CyaA) (reviewed in reference 238). The adenylate cyclases of *M. tuberculosis* are allosterically activated by signals such as low pH (239, 240), bicarbonate/CO_2 (241), and saturated fatty acids (242), which occur during the course of infection.

While cAMP concentrations do not fluctuate with the addition of glucose to the medium, cAMP availability increases ~50-fold during macrophage infection (237). The entry of *M. tuberculosis* into a nonreplicating persister or "quiescent" state during chronic infection requires a significant downshift in metabolism, which is thought to be achieved by diverting acetyl-CoA away from the TCA cycle toward the synthesis of triacylglycerides (243). Mycobacteria accumulate and store triacylglycerides under stress conditions, although their role is not fully understood.

In *M. tuberculosis*, cAMP is integral to the control of the RLA system through the allosteric regulation of the acetyltransferase *Mt*PatA. It is possible that mycobacteria may use cAMP availability to adjust flux through AMP-forming acyl-CoA synthetases, the targets of *Ms*PatA (192). Taken together, it is also conceivable that *M. tuberculosis* would downregulate Acs activity as a mechanism to reduce the available acetyl-CoA pool in order to slow down cellular metabolism during chronic infection.

Cellular Stress and RLA

Mammalian sirtuins have been reported to play a role in cellular protection by promoting positive effects on processes such as DNA repair, cell survival, and stress resistance (reviewed in reference 244). Recent studies have shown that this effect is also conserved in bacteria.

Deletion of the *E. coli* sirtuin deacetylase (*Ec*CobB) increases acetylation levels in the cell, which was shown to increase resistance to both heat and oxidative stress (245). This same study performed whole-transcriptome analysis of a Δ*cobB* strain and found that many stress-related systems were repressed in this mutant, including genes related to heat shock, osmotic stress, acid resistance, cold shock, and carbon starvation (245). These data provided compelling evidence that RLA is a mechanism used by cells to respond to environmental stressors.

A recent acetylome study of *M. tuberculosis* identified that 10% of the enzymes involved in fatty acid biosynthesis were acetylated (143). Fatty acid biosynthesis in this organism has been shown to play a role in colony morphology and biofilm formation. The authors of that study demonstrated that deletion of the sirtuin deacetylase in this organism (MRA_1161) resulted in a more granular morphology, a decrease in biofilm formation, and increased resistance to heat stress (143). These studies further support the hypothesis that RLA is a mechanism for protection against environmental stresses.

TRANSCRIPTIONAL REGULATION OF GENES ENCODING RLA ENZYMES

While the field is beginning to identify acetyltransferases, partner deacetylases, and protein targets as well as to distinguish structural determinants needed for recognition and enzyme regulation, much remains unknown about the transcriptional regulation of the genes encoding the components of the RLA systems.

Regulation of RLA Genes in *Escherichia coli*

The *pka* gene, which encodes the protein acetyltransferase of *E. coli*, has a similar expression profile to that of *acs*, one of its target substrates (177). When *E. coli* grows under certain conditions (i.e., glucose minimal medium), the TCA cycle cannot process acetyl-CoA quickly enough and instead utilizes an overflow pathway to excrete acetate. This acetate pool is later assimilated via Acs and utilized by the cell when glucose is depleted (246).

This "acetate switch" is mediated in part by the expression level of *acs*, which is low during exponential phase but increases dramatically in late exponential and early stationary phases (247). Expression of *acs* is controlled by several transcription factors, including the nucleoid proteins Fis and integration host factor (IHF) as well as Crp (177, 248). Interestingly, the *E. coli* protein acetyltransferase gene *pka* has an expression profile similar to that of its target, *acs*, during growth on glucose (177). Transcription of *pka* is also activated by Crp in response to cAMP levels (177). The activation of *pka* and *acs* expression by Crp may represent a regulatory mimic of the direct cAMP-dependent activation of GNAT activity observed in mycobacteria. At present, it appears that the *cobB* gene encoding the sirtuin in *E. coli* is constitutively expressed and is not dependent on cAMP levels (177).

Multiple Isoforms of the CobB Deacetylase Are Present in *Salmonella enterica*

In *S. enterica*, the CobB sirtuin exists as two isoforms, due to the presence of two independent start codons that are read in the same open reading frame. This results in the production of a long isoform ($CobB_L$) (273 amino acids [aa]) and a short isoform ($CobB_S$) (236 aa), which lacks 37 of the amino acids present in $CobB_L$ (249). Interestingly, both the $CobB_L$ and $CobB_S$ isoforms are active, although $CobB_S$ is the dominant isoform *in vivo* and is produced at ~10-fold-higher levels (249). The presence of the dual start codons is not limited to *S. enterica* and is found throughout the enterobacteria. The physiological relevance of $CobB_L$ and $CobB_S$ and their contribution to RLA are currently unknown.

CONCLUSIONS

RLA is an emerging field in prokaryotes that is advancing by leaps and bounds through the use of high-throughput and detailed

mechanistic studies in a variety of organisms. Validation of ideas obtained through global "omics" approaches is key to improving our understanding of the role of RLA. GNAT protein acetyltransferases and their cognate protein deacetylases have been identified in bacteria, archaea, and eukaryotes. The abundance of GNATs in cells of all domains of life is a strong indicator of the relevance of these enzymes to life. Although the physiological role of the majority of these enzymes remains unknown, and the elucidation of their function is a challenge to cell physiologists, efforts to advance this research area will likely provide valuable insights into the strategies used by cells to cope with metabolic stress.

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ADDENDUM IN PROOF

In a study accepted for publication after our manuscript was written [K. L. Hentchel, S. Thao, P. J. Intile, and J. C. Escalante-Semerena, mBio **6**(4):e00891-15, 2015, in press], the authors report insights into the regulatory circuitry that integrates the expression of genes encoding the protein acetyltransferase, the sirtuin deacetylase, and the acetyl-CoA synthetase enzymes of *Salmonella enterica*.

REFERENCES

1. **Walsh CT, Garneau-Tsodikova S, Gatto GJ, Jr.** 2005. Protein post-translational modifications: the chemistry of proteome diversifications. Angew Chem Int Ed Engl **44:**7342–7372. http://dx.doi.org/10.1002/anie.200501023.
2. **Phillips DM.** 1963. The presence of acetyl groups of histones. Biochem J **87:**258–263.
3. **Jarrell KF, Ding Y, Meyer BH, Albers SV, Kaminski L, Eichler J.** 2014. N-linked glycosylation in archaea: a structural, functional, and genetic analysis. Microbiol Mol Biol Rev **78:**304–341. http://dx.doi.org/10.1128/MMBR.00052-13.
4. **Zverina EA, Lamphear CL, Wright EN, Fierke CA.** 2012. Recent advances in protein prenyltransferases: substrate identification, regulation, and disease interventions. Curr Opin Chem Biol **16:**544–552. http://dx.doi.org/10.1016/j.cbpa.2012.10.015.
5. **Rivera C, Gurard-Levin ZA, Almouzni G, Loyola A.** 2014. Histone lysine methylation and chromatin replication. Biochim Biophys Acta **1839:**1433–1439. http://dx.doi.org/10.1016/j.bbagrm.2014.03.009.
6. **Gould N, Doulias PT, Tenopoulou M, Raju K, Ischiropoulos H.** 2013. Regulation of protein function and signaling by reversible cysteine S-nitrosylation. J Biol Chem **288:**26473–26479. http://dx.doi.org/10.1074/jbc.R113.460261.
7. **Kennelly PJ.** 2014. Protein Ser/Thr/Tyr phosphorylation in the Archaea. J Biol Chem **289:**9480–9487. http://dx.doi.org/10.1074/jbc.R113.529412.
8. **Cousin C, Derouiche A, Shi L, Pagot Y, Poncet S, Mijakovic I.** 2013. Protein-serine/threonine/tyrosine kinases in bacterial signaling and regulation. FEMS Microbiol Lett **346:**11–19. http://dx.doi.org/10.1111/1574-6968.12189.
9. **Weinert BT, Scholz C, Wagner SA, Iesmantavicius V, Su D, Daniel JA, Choudhary C.** 2013. Lysine succinylation is a frequently occurring modification in prokaryotes and eukaryotes and extensively overlaps with acetylation. Cell Rep **4:**842–851. http://dx.doi.org/10.1016/j.celrep.2013.07.024.
10. **Rieser E, Cordier SM, Walczak H.** 2013. Linear ubiquitination: a newly discovered regulator of cell signalling. Trends Biochem Sci **38:**94–102. http://dx.doi.org/10.1016/j.tibs.2012.11.007.
11. **Muller MP, Albers MF, Itzen A, Hedberg C.** 2014. Exploring adenylylation and phosphocholination as post-translational modifications. Chembiochem **15:**19–26. http://dx.doi.org/10.1002/cbic.201300508.
12. **Van Meter M, Mao Z, Gorbunova V, Seluanov A.** 2011. Repairing split ends: SIRT6, mono-ADP ribosylation and DNA repair. Aging (Albany NY) **3:**829–835.
13. **Paquette N, Conlon J, Sweet C, Rus F, Wilson L, Pereira A, Rosadini CV, Goutagny N, Weber AN, Lane WS, Shaffer SA, Maniatis S, Fitzgerald KA, Stuart L, Silverman N.** 2012. Serine/threonine acetylation of TGFbeta-activated kinase (TAK1) by Yersinia pestis YopJ inhibits innate immune signaling. Proc Natl Acad Sci U S A **109:**12710–12715. http://dx.doi.org/10.1073/pnas.1008203109.
14. **Stuecker TN, Hodge KM, Escalante-Semerena JC.** 2012. The missing link in coenzyme A biosynthesis: PanM (formerly YhhK), a yeast GCN5 acetyltransferase homologue triggers aspartate decarboxylase (PanD) maturation in *Salmonella enterica*. Mol Microbiol **84:**608–619. http://dx.doi.org/10.1111/j.1365-2958.2012.08046.x.
15. **Stuecker TN, Tucker AC, Escalante-Semerena JC.** 2012. PanM, an acetyl-coenzyme A sensor required for maturation of L-aspartate decarboxylase (PanD). mBio **3**(4):e00158-12. http://dx.doi.org/10.1128/mBio.00158-12.
16. **Cui W, Shi Z, Fang Y, Zhou L, Ding N, Zhou Z.** 2014. Significance of Arg3, Arg54, and Tyr58 of L-aspartate alpha-decarboxylase from *Corynebacterium glutamicum* in the process of self-cleavage. Biotechnol Lett **36:**121–126. http://dx.doi.org/10.1007/s10529-013-1337-9.
17. **Helbig AO, Gauci S, Raijmakers R, van Breukelen B, Slijper M, Mohammed S, Heck AJ.** 2010. Profiling of *N*-acetylated protein termini provides in-depth insights into the N-terminal nature of the proteome. Mol Cell Proteomics **9:**928–939. http://dx.doi.org/10.1074/mcp.M900463-MCP200.
18. **Helsens K, Van Damme P, Degroeve S, Martens L, Arnesen T, Vandekerckhove J, Gevaert K.** 2011. Bioinformatics analysis of a *Saccharomyces cerevisiae* N-terminal proteome provides evidence of alternative translation initiation and post-translational N-terminal acetylation. J Proteome Res **10:**3578–3589. http://dx.doi.org/10.1021/pr2002325.
19. **Sterner DE, Berger SL.** 2000. Acetylation of histones and transcription-related factors. Microbiol Mol Biol Rev **64:**435–459. http://dx.doi.org/10.1128/MMBR.64.2.435-459.2000.
20. **Khorasanizadeh S.** 2004. The nucleosome: from genomic organization to genomic regulation. Cell **116:**259–272. http://dx.doi.org/10.1016/S0092-8674(04)00044-3.
21. **Gu W, Roeder RG.** 1997. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell **90:**595–606. http://dx.doi.org/10.1016/S0092-8674(00)80521-8.
22. **Yang XJ, Seto E.** 2008. Lysine acetylation: codified crosstalk with other posttranslational modifications. Mol Cell **31:**449–461. http://dx.doi.org/10.1016/j.molcel.2008.07.002.
23. **Starai VJ, Celic I, Cole RN, Boeke JD, Escalante-Semerena JC.** 2002. Sir2-dependent activation of acetyl-CoA synthetase by deacetylation of active lysine. Science **298:**2390–2392. http://dx.doi.org/10.1126/science.1077650.
24. **Starai VJ, Escalante-Semerena JC.** 2004. Identification of the protein acetyltransferase (Pat) enzyme that acetylates acetyl-CoA synthetase in *Salmonella enterica*. J Mol Biol **340:**1005–1012. http://dx.doi.org/10.1016/j.jmb.2004.05.010.
25. **Soppa J.** 2010. Protein acetylation in archaea, bacteria, and eukaryotes. Archaea **2010:**pii=820681. http://dx.doi.org/10.1155/2010/820681.
26. **Kouzarides T.** 2007. Chromatin modifications and their function. Cell **128:**693–705. http://dx.doi.org/10.1016/j.cell.2007.02.005.
27. **Glozak MA, Sengupta N, Zhang X, Seto E.** 2005. Acetylation and deacetylation of non-histone proteins. Gene **363:**15–23. http://dx.doi.org/10.1016/j.gene.2005.09.010.
28. **Crosby HA, Pelletier DA, Hurst GB, Escalante-Semerena JC.** 2012. System-wide studies of N-lysine acetylation in *Rhodopseudomonas palustris* reveal substrate specificity of protein acetyltransferases. J Biol Chem **287:**15590–15601. http://dx.doi.org/10.1074/jbc.M112.352104.
29. **Starai VJ, Takahashi H, Boeke JD, Escalante-Semerena JC.** 2003. Short-chain fatty acid activation by acyl-coenzyme A synthetases requires SIR2 protein function in *Salmonella enterica* and *Saccharomyces cerevisiae*. Genetics **163:**545–555.
30. **Gardner JG, Grundy FJ, Henkin TM, Escalante-Semerena JC.** 2006. Control of acetyl-coenzyme A synthetase (AcsA) activity by acetylation/deacetylation without NAD(+) involvement in *Bacillus subtilis*. J Bacteriol **188:**5460–5468. http://dx.doi.org/10.1128/JB.00215-06.
31. **Tucker AC, Escalante-Semerena JC.** 2013. Acetoacetyl-CoA synthetase activity is controlled by a protein acetyltransferase with unique domain organization in *Streptomyces lividans*. Mol Microbiol **87:**152–167. http://dx.doi.org/10.1111/mmi.12088.
32. **Hubbert C, Guardiola A, Shao R, Kawaguchi Y, Ito A, Nixon A, Yoshida M, Wang XF, Yao TP.** 2002. HDAC6 is a microtubule-associated deacetylase. Nature **417:**455–458. http://dx.doi.org/10.1038/417455a.

33. **Kim GW, Yang XJ.** 2011. Comprehensive lysine acetylomes emerging from bacteria to humans. Trends Biochem Sci **36:**211–220. http://dx.doi.org/10.1016/j.tibs.2010.10.001.
34. **Xiong Y, Guan KL.** 2012. Mechanistic insights into the regulation of metabolic enzymes by acetylation. J Cell Biol **198:**155–164. http://dx.doi.org/10.1083/jcb.201202056.
35. **Lin YY, Lu JY, Zhang J, Walter W, Dang W, Wan J, Tao SC, Qian J, Zhao Y, Boeke JD, Berger SL, Zhu H.** 2009. Protein acetylation microarray reveals that NuA4 controls key metabolic target regulating gluconeogenesis. Cell **136:**1073–1084. http://dx.doi.org/10.1016/j.cell.2009.01.033.
36. **Lv L, Li D, Zhao D, Lin R, Chu Y, Zhang H, Zha Z, Liu Y, Li Z, Xu Y, Wang G, Huang Y, Xiong Y, Guan KL, Lei QY.** 2011. Acetylation targets the M2 isoform of pyruvate kinase for degradation through chaperone-mediated autophagy and promotes tumor growth. Mol Cell **42:**719–730. http://dx.doi.org/10.1016/j.molcel.2011.04.025.
37. **Hallows WC, Yu W, Denu JM.** 2012. Regulation of glycolytic enzyme phosphoglycerate mutase-1 by Sirt1 protein-mediated deacetylation. J Biol Chem **287:**3850–3858. http://dx.doi.org/10.1074/jbc.M111.317404.
38. **LeDizet M, Piperno G.** 1987. Identification of an acetylation site of *Chlamydomonas* alpha-tubulin. Proc Natl Acad Sci U S A **84:**5720–5724. http://dx.doi.org/10.1073/pnas.84.16.5720.
39. **Kouzarides T.** 2000. Acetylation: a regulatory modification to rival phosphorylation? EMBO J **19:**1176–1179. http://dx.doi.org/10.1093/emboj/19.6.1176.
40. **Tanner KG, Langer MR, Denu JM.** 2000. Kinetic mechanism of human histone acetyltransferase P/CAF. Biochemistry **39:**11961–11969. http://dx.doi.org/10.1021/bi001272h.
41. **Tanner KG, Langer MR, Kim Y, Denu JM.** 2000. Kinetic mechanism of the histone acetyltransferase GCN5 from yeast. J Biol Chem **275:**22048–22055. http://dx.doi.org/10.1074/jbc.M002893200.
42. **Marmorstein R, Roth SY.** 2001. Histone acetyltransferases: function, structure, and catalysis. Curr Opin Genet Dev **11:**155–161. http://dx.doi.org/10.1016/S0959-437X(00)00173-8.
43. **Berndsen CE, Albaugh BN, Tan S, Denu JM.** 2007. Catalytic mechanism of a MYST family histone acetyltransferase. Biochemistry **46:**623–629. http://dx.doi.org/10.1021/bi602513x.
44. **Thompson PR, Kurooka H, Nakatani Y, Cole PA.** 2001. Transcriptional coactivator protein p300. Kinetic characterization of its histone acetyltransferase activity. J Biol Chem **276:**33721–33729.
45. **Yan Y, Barlev NA, Haley RH, Berger SL, Marmorstein R.** 2000. Crystal structure of yeast Esa1 suggests a unified mechanism for catalysis and substrate binding by histone acetyltransferases. Mol Cell **6:**1195–1205. http://dx.doi.org/10.1016/S1097-2765(00)00116-7.
46. **Yan Y, Harper S, Speicher DW, Marmorstein R.** 2002. The catalytic mechanism of the ESA1 histone acetyltransferase involves a self-acetylated intermediate. Nat Struct Biol **9:**862–869.
47. **Liu X, Wang L, Zhao K, Thompson PR, Hwang Y, Marmorstein R, Cole PA.** 2008. The structural basis of protein acetylation by the p300/CBP transcriptional coactivator. Nature **451:**846–850. http://dx.doi.org/10.1038/nature06546.
48. **Wang L, Tang Y, Cole PA, Marmorstein R.** 2008. Structure and chemistry of the p300/CBP and Rtt109 histone acetyltransferases: implications for histone acetyltransferase evolution and function. Curr Opin Struct Biol **18:**741–747. http://dx.doi.org/10.1016/j.sbi.2008.09.004.
49. **Rojas JR, Trievel RC, Zhou J, Mo Y, Li X, Berger SL, Allis CD, Marmorstein R.** 1999. Structure of *Tetrahymena* GCN5 bound to coenzyme A and a histone H3 peptide. Nature **401:**93–98. http://dx.doi.org/10.1038/43487.
50. **Dutnall RN, Tafrov ST, Sternglanz R, Ramakrishnan V.** 1998. Structure of the histone acetyltransferase Hat1: a paradigm for the GCN5-related N-acetyltransferase superfamily. Cell **94:**427–438. http://dx.doi.org/10.1016/S0092-8674(00)81584-6.
51. **Clements A, Rojas JR, Trievel RC, Wang L, Berger SL, Marmorstein R.** 1999. Crystal structure of the histone acetyltransferase domain of the human PCAF transcriptional regulator bound to coenzyme A. EMBO J **18:**3521–3532.
52. **Lin Y, Fletcher CM, Zhou J, Allis CD, Wagner G.** 1999. Solution structure of the catalytic domain of GCN5 histone acetyltransferase bound to coenzyme A. Nature **400:**86–89.
53. **Vetting MW, de Carvalho LPS, Yu M, Hegde SS, Magnet S, Roderick SL, Blanchard JS.** 2005. Structure and functions of the GNAT superfamily of acetyltransferases. Arch Biochem Biophys **433:**212–226. http://dx.doi.org/10.1016/j.abb.2004.09.003.
54. **Dyda F, Klein DC, Hickman AB.** 2000. GCN5-related N-acetyltransferases: a structural overview. Annu Rev Biophys Biomol Struct **29:**81–103. http://dx.doi.org/10.1146/annurev.biophys.29.1.81.
55. **Hodawadekar SC, Marmorstein R.** 2007. Chemistry of acetyl transfer by histone modifying enzymes: structure, mechanism and implications for effector design. Oncogene **26:**5528–5540. http://dx.doi.org/10.1038/sj.onc.1210619.
56. **Garrity J, Gardner JG, Hawse W, Wolberger C, Escalante-Semerena JC.** 2007. N-Lysine propionylation controls the activity of propionyl-CoA synthetase. J Biol Chem **282:**30239–30245. http://dx.doi.org/10.1074/jbc.M704409200.
57. **Cheng Z, Tang Y, Chen Y, Kim S, Liu H, Li SS, Gu W, Zhao Y.** 2009. Molecular characterization of propionyllysines in non-histone proteins. Mol Cell Proteomics **8:**45–52. http://dx.doi.org/10.1074/mcp.M800224-MCP200.
58. **Berndsen CE, Denu JM.** 2008. Catalysis and substrate selection by histone/protein lysine acetyltransferases. Curr Opin Struct Biol **18:**682–689. http://dx.doi.org/10.1016/j.sbi.2008.11.004.
59. **Marsh VL, Peak-Chew SY, Bell SD.** 2005. Sir2 and the acetyltransferase, Pat, regulate the archaeal chromatin protein, Alba. J Biol Chem **280:**21122–21228. http://dx.doi.org/10.1074/jbc.M501280200.
60. **Thao S, Escalante-Semerena JC.** 2011. Control of protein function by reversible N(epsilon)-lysine acetylation in bacteria. Curr Opin Microbiol **14:**200–204. http://dx.doi.org/10.1016/j.mib.2010.12.013.
61. **Wright GD, Ladak P.** 1997. Overexpression and characterization of the chromosomal aminoglycoside 6′-N-acetyltransferase from *Enterococcus faecium*. Antimicrob Agents Chemother **41:**956–960.
62. **Wolf E, Vassilev A, Makino Y, Sali A, Nakatani Y, Burley SK.** 1998. Crystal structure of a GCN5-related N-acetyltransferase: *Serratia marcescens* aminoglycoside 3-N-acetyltransferase. Cell **94:**439–449. http://dx.doi.org/10.1016/S0092-8674(00)81585-8.
63. **Davies J, Wright GD.** 1997. Bacterial resistance to aminoglycoside antibiotics. Trends Microbiol **5:**234–240. http://dx.doi.org/10.1016/S0966-842X(97)01033-0.
64. **Chen Y, Sprung R, Tang Y, Ball H, Sangras B, Kim S, Falck JR, Peng J, Gu W, Zhao Y.** 2007. Lysine propionylation and butyrylation are novel post-translational modifications in histones. Mol Cell Proteomics **6:**812–819. http://dx.doi.org/10.1074/mcp.M700021-MCP200.
65. **Leemhuis H, Packman LC, Nightingale KP, Hollfelder F.** 2008. The human histone acetyltransferase P/CAF is a promiscuous histone propionyltransferase. Chembiochem **9:**499–503. http://dx.doi.org/10.1002/cbic.200700556.
66. **Feng L, Wang W, Cheng J, Ren Y, Zhao G, Gao C, Tang Y, Liu X, Han W, Peng X, Liu R, Wang L.** 2007. Genome and proteome of long-chain alkane degrading *Geobacillus thermodenitrificans* NG80-2 isolated from a deep-subsurface oil reservoir. Proc Natl Acad Sci U S A **104:**5602–5607. http://dx.doi.org/10.1073/pnas.0609650104.
67. **Neuwald AF, Landsman D.** 1997. GCN5-related histone N-acetyltransferases belong to a diverse superfamily that includes the yeast SPT10 protein. Trends Biochem Sci **22:**154–155. http://dx.doi.org/10.1016/S0968-0004(97)01034-7.
68. **Liang W, Deutscher MP.** 2012. Post-translational modification of RNase R is regulated by stress-dependent reduction in the acetylating enzyme Pka (YfiQ). RNA **18:**37–41. http://dx.doi.org/10.1261/rna.030213.111.
69. **Crosby HA, Heiniger EK, Harwood CS, Escalante-Semerena JC.** 2010. Reversible N(epsilon)-lysine acetylation regulates the activity of acyl-CoA synthetases involved in anaerobic benzoate catabolism in *Rhodopseudomonas palustris*. Mol Microbiol **76:**874–888. http://dx.doi.org/10.1111/j.1365-2958.2010.07127.x.
70. **Thao S, Escalante-Semerena JC.** 2012. A positive selection approach identifies residues important for folding of *Salmonella enterica* Pat, an N(epsilon)-lysine acetyltransferase that regulates central metabolism enzymes. Res Microbiol **163:**427–435. http://dx.doi.org/10.1016/j.resmic.2012.05.008.
71. **Tucker AC, Taylor KC, Rank KC, Rayment I, Escalante-Semerena JC.** 2014. Insights into the specificity of lysine acetyltransferases. J Biol Chem **289:**36249–36262. http://dx.doi.org/10.1074/jbc.M114.613901.
72. **Thao S, Escalante-Semerena JC.** 2011. Biochemical and thermodynamic analyses of *Salmonella enterica* Pat, a multidomain, multimeric N(epsi-

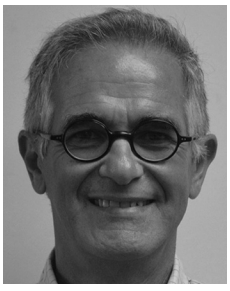
lon)-lysine acetyltransferase involved in carbon and energy metabolism. mBio 2(5):e00216-11. http://dx.doi.org/10.1128/mBio.00216-11.
73. **Mackay DT, Botting CH, Taylor GL, White MF.** 2007. An acetylase with relaxed specificity catalyses protein N-terminal acetylation in *Sulfolobus solfataricus*. Mol Microbiol **64:**1540–1548. http://dx.doi.org/10.1111/j.1365-2958.2007.05752.x.
74. **Brent MM, Iwata A, Carten J, Zhao K, Marmorstein R.** 2009. Structure and biochemical characterization of protein acetyltransferase from *Sulfolobus solfataricus*. J Biol Chem **284:**19412–19419. http://dx.doi.org/10.1074/jbc.M109.014951.
75. **Glasemacher J, Bock AK, Schmid R, Schönheit P.** 1997. Purification and properties of acetyl-CoA synthetase (ADP-forming), an archaeal enzyme of acetate formation and ATP synthesis, from the hyperthermophile *Pyrococcus furiosus*. Eur J Biochem **244:**561–567. http://dx.doi.org/10.1111/j.1432-1033.1997.00561.x.
76. **Shikata K, Fukui T, Atomi H, Imanaka T.** 2007. A novel ADP-forming succinyl-CoA synthetase in *Thermococcus kodakaraensis* structurally related to the archaeal nucleoside diphosphate-forming acetyl-CoA synthetases. J Biol Chem **282:**26963–26970. http://dx.doi.org/10.1074/jbc.M702694200.
77. **Sánchez LB, Galperin MY, Muller M.** 2000. Acetyl-CoA synthetase from the amitochondriate eukaryote Giardia lamblia belongs to the newly recognized superfamily of acyl-CoA synthetases (nucleoside diphosphate-forming). J Biol Chem **275:**5794–5803. http://dx.doi.org/10.1074/jbc.275.8.5794.
78. **Gardner JG, Escalante-Semerena JC.** 2008. Biochemical and mutational analyses of AcuA, the acetyltransferase enzyme that controls the activity of the acetyl coenzyme A synthetase (AcsA) in *Bacillus subtilis*. J Bacteriol **190:**5132–5136. http://dx.doi.org/10.1128/JB.00340-08.
79. **Marsh VL, Peak-Chew SY, Bell SD.** 2005. Sir2 and the acetyltransferase, Pat, regulate the archaeal chromatin protein, Alba. J Biol Chem **280:**21122–21128. http://dx.doi.org/10.1074/jbc.M501280200.
80. **Hu LI, Lima BP, Wolfe AJ.** 2010. Bacterial protein acetylation: the dawning of a new age. Mol Microbiol **77:**15–21. http://dx.doi.org/10.1111/j.1365-2958.2010.07204.x.
81. **Weinert BT, Iesmantavicius V, Wagner SA, Scholz C, Gummesson B, Beli P, Nystrom T, Choudhary C.** 2013. Acetyl-phosphate is a critical determinant of lysine acetylation in *E. coli*. Mol Cell **51:**265–272. http://dx.doi.org/10.1016/j.molcel.2013.06.003.
82. **Cumberlidge AG, Isono K.** 1979. Ribosomal protein modification in Escherichia coli. I. A mutant lacking the N-terminal acetylation of protein S5 exhibits thermosensitivity. J Mol Biol **131:**169–189.
83. **Isono K, Isono S.** 1980. Ribosomal protein modification in *Escherichia coli*. II. Studies of a mutant lacking the N-terminal acetylation of protein S18. Mol Gen Genet **177:**645–651.
84. **Fukuchi J, Kashiwagi K, Takio K, Igarashi K.** 1994. Properties and structure of spermidine acetyltransferase in *Escherichia coli*. J Biol Chem **269:**22581–22585.
85. **Hung MN, Rangarajan E, Munger C, Nadeau G, Sulea T, Matte A.** 2006. Crystal structure of TDP-fucosamine acetyltransferase (WecD) from *Escherichia coli*, an enzyme required for enterobacterial common antigen synthesis. J Bacteriol **188:**5606–5617. http://dx.doi.org/10.1128/JB.00306-06.
86. **Marvil DK, Leisinger T.** 1977. N-Acetylglutamate synthase of *Escherichia coli*: purification, characterization, and molecular properties. J Biol Chem **252:**3295–32303.
87. **Kazakov T, Kuznedelov K, Semenova E, Mukhamedyarov D, Datsenko KA, Metlitskaya A, Vondenhoff GH, Tikhonov A, Agarwal V, Nair S, Van Aerschot A, Severinov K.** 2014. The RimL transacetylase provides resistance to translation inhibitor microcin. C J Bacteriol **196:**3377–3385. http://dx.doi.org/10.1128/JB.01584-14.
88. **Ikeuchi Y, Kitahara K, Suzuki T.** 2008. The RNA acetyltransferase driven by ATP hydrolysis synthesizes N^4-acetylcytidine of tRNA anticodon. EMBO J **27:**2194–2203. http://dx.doi.org/10.1038/emboj.2008.154.
89. **Yoshikawa A, Isono S, Sheback A, Isono K.** 1987. Cloning and nucleotide sequencing of the genes *rimI* and *rimJ* which encode enzymes acetylating ribosomal proteins S18 and S5 of Escherichia coli K12. Mol Gen Genet **209:**481–488. http://dx.doi.org/10.1007/BF00331153.
90. **Tanaka S, Matsushita Y, Yoshikawa A, Isono K.** 1989. Cloning and molecular characterization of the gene *rimL* which encodes an enzyme acetylating ribosomal protein L12 of *Escherichia coli* K12. Mol Gen Genet **217:**289–293. http://dx.doi.org/10.1007/BF02464895.
91. **Hentchel KL, Escalante-Semerena JC.** 2015. In *Salmonella enterica*, the Gcn5-related acetyltransferase MddA (formerly YncA) acetylates methionine sulfoximine and methionine sulfone, blocking their toxic effects. J Bacteriol **197:**314–325. http://dx.doi.org/10.1128/JB.02311-14.
92. **Liang W, Malhotra A, Deutscher MP.** 2011. Acetylation regulates the stability of a bacterial protein: growth stage-dependent modification of RNase R. Mol Cell **44:**160–166. http://dx.doi.org/10.1016/j.molcel.2011.06.037.
93. **Leibowitz MJ, Soffer RL.** 1970. Enzymatic modification of proteins. 3. Purification and properties of a leucyl, phenylalanyl transfer ribonucleic acid protein transferase from *Escherichia coli*. J Biol Chem **245:**2066–2073.
94. **Tobias JW, Shrader TE, Rocap G, Varshavsky A.** 1991. The N-end rule in bacteria. Science **254:**1374–1377. http://dx.doi.org/10.1126/science.1962196.
95. **Nozaki S, Webb ME, Niki H.** 2012. An activator for pyruvoyl-dependent L-aspartate alpha-decarboxylase is conserved in a small group of the gamma-proteobacteria including *Escherichia coli*. Microbiologyopen **1:**298–310. http://dx.doi.org/10.1002/mbo3.34.
96. **Monteiro DC, Rugen MD, Shepherd D, Nozaki S, Niki H, Webb ME.** 2012. Formation of a heterooctameric complex between aspartate alpha-decarboxylase and its cognate activating factor, PanZ, is CoA-dependent. Biochem Biophys Res Commun **426:**350–355. http://dx.doi.org/10.1016/j.bbrc.2012.08.084.
97. **Sauve AA, Wolberger C, Schramm VL, Boeke JD.** 2006. The biochemistry of sirtuins. Annu Rev Biochem **75:**435–465. http://dx.doi.org/10.1146/annurev.biochem.74.082803.133500.
98. **Tsang AW, Escalante-Semerena JC.** 1998. CobB, a new member of the SIR2 family of eucaryotic regulatory proteins, is required to compensate for the lack of nicotinate mononucleotide:5,6-dimethylbenzimidazole phosphoribosyltransferase activity in *cobT* mutants during cobalamin biosynthesis in *Salmonella typhimurium* LT2. J Biol Chem **273:**31788–31794. http://dx.doi.org/10.1074/jbc.273.48.31788.
99. **Frye RA.** 2000. Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. Biochem Biophys Res Commun **273:**793–798. http://dx.doi.org/10.1006/bbrc.2000.3000.
100. **Marmorstein R.** 2001. Structure of histone deacetylases: insights into substrate recognition and catalysis. Structure **9:**1127–1133. http://dx.doi.org/10.1016/S0969-2126(01)00690-6.
101. **Lombardi PM, Cole KE, Dowling DP, Christianson DW.** 2011. Structure, mechanism, and inhibition of histone deacetylases and related metalloenzymes. Curr Opin Struct Biol **21:**735–743. http://dx.doi.org/10.1016/j.sbi.2011.08.004.
102. **Smith BC, Denu JM.** 2007. Acetyl-lysine analog peptides as mechanistic probes of protein deacetylases. J Biol Chem **282:**37256–37265. http://dx.doi.org/10.1074/jbc.M707878200.
103. **Zhou Y, Zhang H, He B, Du J, Lin H, Cerione RA, Hao Q.** 2012. The bicyclic intermediate structure provides insights into the desuccinylation mechanism of human sirtuin 5 (SIRT5). J Biol Chem **287:**28307–28314. http://dx.doi.org/10.1074/jbc.M112.384511.
104. **Park J, Chen Y, Tishkoff DX, Peng C, Tan M, Dai L, Xie Z, Zhang Y, Zwaans BM, Skinner ME, Lombard DB, Zhao Y.** 2013. SIRT5-mediated lysine desuccinylation impacts diverse metabolic pathways. Mol Cell **50:**919–930. http://dx.doi.org/10.1016/j.molcel.2013.06.001.
105. **Peng C, Lu Z, Xie Z, Cheng Z, Chen Y, Tan M, Luo H, Zhang Y, He W, Yang K, Zwaans BM, Tishkoff D, Ho L, Lombard D, He TC, Dai J, Verdin E, Ye Y, Zhao Y.** 2011. The first identification of lysine malonylation substrates and its regulatory enzyme. Mol Cell Proteomics **10:**M111.012658. http://dx.doi.org/10.1074/mcp.M111.012658.
106. **Osborne B, Cooney GJ, Turner N.** 2014. Are sirtuin deacylase enzymes important modulators of mitochondrial energy metabolism? Biochim Biophys Acta **1840:**1295–1302. http://dx.doi.org/10.1016/j.bbagen.2013.08.016.
107. **He W, Newman JC, Wang MZ, Ho L, Verdin E.** 2012. Mitochondrial sirtuins: regulators of protein acylation and metabolism. Trends Endocrinol Metab **23:**467–476. http://dx.doi.org/10.1016/j.tem.2012.07.004.
108. **Lin SJ, Guarente L.** 2003. Nicotinamide adenine dinucleotide, a metabolic regulator of transcription, longevity and disease. Curr Opin Cell Biol **15:**241–246. http://dx.doi.org/10.1016/S0955-0674(03)00006-1.
109. **Denu JM.** 2003. Linking chromatin function with metabolic networks: Sir2 family of NAD(+)-dependent deacetylases. Trends Biochem Sci **28:**41–48. http://dx.doi.org/10.1016/S0968-0004(02)00005-1.
110. **Moazed D.** 2001. Enzymatic activities of Sir2 and chromatin silencing.

Curr Opin Cell Biol **13:**232–238. http://dx.doi.org/10.1016/S0955-0674(00)00202-7.
111. **Blander G, Guarente L.** 2004. The sir2 family of protein deacetylases. Annu Rev Biochem **73:**417–435. http://dx.doi.org/10.1146/annurev.biochem.73.011303.073651.
112. **Rowen JW, Kornberg A.** 1951. The phosphorolysis of nicotinamide riboside. J Biol Chem **193:**497–507.
113. **Zhao K, Harshaw R, Chai X, Marmorstein R.** 2004. Structural basis for nicotinamide cleavage and ADP-ribose transfer by NAD+-dependent Sir2 histone/protein deacetylases. Proc Natl Acad Sci U S A **101:**8563–8568. http://dx.doi.org/10.1073/pnas.0401057101.
114. **Sauve AA, Celic I, Avalos J, Deng H, Boeke JD, Schramm VL.** 2001. Chemistry of gene silencing: the mechanism of NAD(+)-dependent deacetylation reactions. Biochemistry **40:**15456–15463. http://dx.doi.org/10.1021/bi011858j.
115. **Smith JS, Brachmann CB, Celic I, Kenna MA, Muhammad S, Starai VJ, Avalos JL, Escalante-Semerena JC, Grubmeyer C, Wolberger C, Boeke JD.** 2000. A phylogenetically conserved NAD^+-dependent protein deacetylase activity in the Sir2 protein family. Proc Natl Acad Sci U S A **97:**6658–6663. http://dx.doi.org/10.1073/pnas.97.12.6658.
116. **Tanner KG, Landry J, Sternglanz R, Denu JM.** 2000. Silent information regulator family of NAD-dependent histone/protein deacetylases generates a unique product, 1-*O*-acetyl-ADP-ribose. Proc Natl Acad Sci U S A **97:**14178–14182. http://dx.doi.org/10.1073/pnas.250422697.
117. **Sauve AA.** 2010. Sirtuin chemical mechanisms. Biochim Biophys Acta **1804:**1591–1603. http://dx.doi.org/10.1016/j.bbapap.2010.01.021.
118. **Borra MT, O'Neill FJ, Jackson MD, Marshall B, Verdin E, Foltz KR, Denu JM.** 2002. Conserved enzymatic production and biological effect of *O*-acetyl-ADP-ribose by silent information regulator 2-like NAD^+-dependent deacetylases. J Biol Chem **277:**12632–12641. http://dx.doi.org/10.1074/jbc.M111830200.
119. **Jackson MD, Denu JM.** 2002. Structural identification of 2′- and 3′-*O*-acetyl-ADP-ribose as novel metabolites derived from the Sir2 family of beta-NAD^+-dependent histone/protein deacetylases. J Biol Chem **277:**18535–18544. http://dx.doi.org/10.1074/jbc.M200671200.
120. **Grubisha O, Rafty LA, Takanishi CL, Xu X, Tong L, Perraud AL, Scharenberg AM, Denu JM.** 2006. Metabolite of SIR2 reaction modulates TRPM2 ion channel. J Biol Chem **281:**14057–14065. http://dx.doi.org/10.1074/jbc.M513741200.
121. **Rafty LA, Schmidt MT, Perraud AL, Scharenberg AM, Denu JM.** 2002. Analysis of *O*-acetyl-ADP-ribose as a target for Nudix ADP-ribose hydrolases. J Biol Chem **277:**47114–47122. http://dx.doi.org/10.1074/jbc.M208997200.
122. **Ono T, Kasamatsu A, Oka S, Moss J.** 2006. The 39-kDa poly(ADP-ribose) glycohydrolase ARH3 hydrolyzes *O*-acetyl-ADP-ribose, a product of the Sir2 family of acetyl-histone deacetylases. Proc Natl Acad Sci U S A **103:**16687–166891. http://dx.doi.org/10.1073/pnas.0607911103.
123. **Schmidt MT, Smith BC, Jackson MD, Denu JM.** 2004. Coenzyme specificity of Sir2 protein deacetylases: implications for physiological regulation. J Biol Chem **279:**40122–40129. http://dx.doi.org/10.1074/jbc.M407484200.
124. **Guarente L.** 2005. Calorie restriction and SIR2 genes—towards a mechanism. Mech Ageing Dev **126:**923–928. http://dx.doi.org/10.1016/j.mad.2005.03.013.
125. **Porcu M, Chiarugi A.** 2005. The emerging therapeutic potential of sirtuin-interacting drugs: from cell death to lifespan extension. Trends Pharmacol Sci **26:**94–103. http://dx.doi.org/10.1016/j.tips.2004.12.009.
126. **Bitterman KJ, Anderson RM, Cohen HY, Latorre-Esteves M, Sinclair DA.** 2002. Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast sir2 and human SIRT1. J Biol Chem **277:**45099–45107. http://dx.doi.org/10.1074/jbc.M205670200.
127. **Avalos JL, Bever KM, Wolberger C.** 2005. Mechanism of sirtuin inhibition by nicotinamide: altering the NAD(+) cosubstrate specificity of a Sir2 enzyme. Mol Cell **17:**855–868. http://dx.doi.org/10.1016/j.molcel.2005.02.022.
128. **Sauve AA, Schramm VL.** 2003. Sir2 regulation by nicotinamide results from switching between base exchange and deacetylation chemistry. Biochemistry **42:**9249–9256. http://dx.doi.org/10.1021/bi034959l.
129. **Jackson MD, Schmidt MT, Oppenheimer NJ, Denu JM.** 2003. Mechanism of nicotinamide inhibition and transglycosidation by Sir2 histone/protein deacetylases. J Biol Chem **278:**50985–50998. http://dx.doi.org/10.1074/jbc.M306552200.
130. **Wimpenny JWT, Firth A.** 1972. Levels of nicotinamide adenine dinucleotide and reduced nicotinamide adenine dinucleotide in facultative bacteria and the effect of oxygen. J Bacteriol **111:**24–32.
131. **Lin H, Vadali RV, Bennett GN, San KY.** 2004. Increasing the acetyl-CoA pool in the presence of overexpressed phosphoenolpyruvate carboxylase or pyruvate carboxylase enhances succinate production in *Escherichia coli*. Biotechnol Prog **20:**1599–1604. http://dx.doi.org/10.1021/bp049843a.
132. **Fulco M, Schiltz RL, Iezzi S, King MT, Zhao P, Kashiwaya Y, Hoffman E, Veech RL, Sartorelli V.** 2003. Sir2 regulates skeletal muscle differentiation as a potential sensor of the redox state. Mol Cell **12:**51–62. http://dx.doi.org/10.1016/S1097-2765(03)00226-0.
133. **Yuan H, Marmorstein R.** 2012. Structural basis for sirtuin activity and inhibition. J Biol Chem **287:**42428–42435. http://dx.doi.org/10.1074/jbc.R112.372300.
134. **Sanders BD, Jackson B, Marmorstein R.** 2010. Structural basis for sirtuin function: what we know and what we don't. Biochim Biophys Acta **1804:**1604–1616. http://dx.doi.org/10.1016/j.bbapap.2009.09.009.
135. **Chakrabarty SP, Balaram H.** 2010. Reversible binding of zinc in *Plasmodium falciparum* Sir2: structure and activity of the apoenzyme. Biochim Biophys Acta **1804:**1743–1750. http://dx.doi.org/10.1016/j.bbapap.2010.06.010.
136. **Iwabata H, Yoshida M, Komatsu Y.** 2005. Proteomic analysis of organ-specific post-translational lysine-acetylation and -methylation in mice by use of anti-acetyllysine and -methyllysine mouse monoclonal antibodies. Proteomics **5:**4653–4664. http://dx.doi.org/10.1002/pmic.200500042.
137. **Kim SC, Sprung R, Chen Y, Xu Y, Ball H, Pei J, Cheng T, Kho Y, Xiao H, Xiao L, Grishin NV, White M, Yang XJ, Zhao Y.** 2006. Substrate and functional diversity of lysine acetylation revealed by a proteomics survey. Mol Cell **23:**607–618. http://dx.doi.org/10.1016/j.molcel.2006.06.026.
138. **Schwer B, Eckersdorff M, Li Y, Silva JC, Fermin D, Kurtev MV, Giallourakis C, Comb MJ, Alt FW, Lombard DB.** 2009. Calorie restriction alters mitochondrial protein acetylation. Aging Cell **8:**604–606. http://dx.doi.org/10.1111/j.1474-9726.2009.00503.x.
139. **Zhao S, Xu W, Jiang W, Yu W, Lin Y, Zhang T, Yao J, Zhou L, Zeng Y, Li H, Li Y, Shi J, An W, Hancock SM, He F, Qin L, Chin J, Yang P, Chen X, Lei Q, Xiong Y, Guan KL.** 2010. Regulation of cellular metabolism by protein lysine acetylation. Science **327:**1000–1004. http://dx.doi.org/10.1126/science.1179689.
140. **Falb M, Aivaliotis M, Garcia-Rizo C, Bisle B, Tebbe A, Klein C, Konstantinidis K, Siedler F, Pfeiffer F, Oesterhelt D.** 2006. Archaeal N-terminal protein maturation commonly involves N-terminal acetylation: a large-scale proteomics survey. J Mol Biol **362:**915–924. http://dx.doi.org/10.1016/j.jmb.2006.07.086.
141. **Yu BJ, Kim JA, Moon JH, Ryu SE, Pan JG.** 2008. The diversity of lysine-acetylated proteins in *Escherichia coli*. J Microbiol Biotechnol **18:**1529–1536.
142. **Zhang J, Sprung R, Pei J, Tan X, Kim S, Zhu H, Liu CF, Grishin NV, Zhao Y.** 2009. Lysine acetylation is a highly abundant and evolutionarily conserved modification in Escherichia coli. Mol Cell Proteomics **8:**215–225. http://dx.doi.org/10.1074/mcp.M800187-MCP200.
143. **Liu F, Yang M, Wang X, Yang S, Gu J, Zhou J, Zhang XE, Deng J, Ge F.** 2014. Acetylome analysis reveals diverse functions of lysine acetylation in *Mycobacterium tuberculosis*. Mol Cell Proteomics **12:**3352–3366. http://dx.doi.org/10.1074/mcp.M114.041962.
144. **Xing S, Poirier Y.** 2012. The protein acetylome and the regulation of metabolism. Trends Plant Sci **17:**423–430. http://dx.doi.org/10.1016/j.tplants.2012.03.008.
145. **Miao J, Lawrence M, Jeffers V, Zhao F, Parker D, Ge Y, Sullivan WJ, Jr, Cui L.** 2013. Extensive lysine acetylation occurs in evolutionarily conserved metabolic pathways and parasite-specific functions during Plasmodium falciparum intraerythrocytic development. Mol Microbiol **89:**660–675. http://dx.doi.org/10.1111/mmi.12303.
146. **Jeffers V, Sullivan WJ, Jr.** 2012. Lysine acetylation is widespread on proteins of diverse function and localization in the protozoan parasite Toxoplasma gondii. Eukaryot Cell **11:**735–742. http://dx.doi.org/10.1128/EC.00088-12.
147. **Choudhary C, Kumar C, Gnad F, Nielsen ML, Rehman M, Walther T, Olsen JV, Mann M.** 2009. Lysine acetylation targets protein complexes and co-regulates major cellular functions. Science **325:**834–840. http://dx.doi.org/10.1126/science.1175371.
148. **Zhu H, Klemic JF, Chang S, Bertone P, Casamayor A, Klemic KG, Smith D, Gerstein M, Reed MA, Snyder M.** 2000. Analysis of yeast

protein kinases using protein chips. Nat Genet **26:**283–289. http://dx.doi.org/10.1038/81576.
149. **Thao S, Chen CS, Zhu H, Escalante-Semerena JC.** 2010. N(epsilon)-Lysine acetylation of a bacterial transcription factor inhibits its DNA-binding activity. PLoS One **5:**e15123. http://dx.doi.org/10.1371/journal.pone.0015123.
150. **Chen Y, Colak G, Zhao Y.** 2013. SILAC-based quantification of Sirt1-responsive lysine acetylome. Methods Mol Biol **1077:**105–120. http://dx.doi.org/10.1007/978-1-62703-637-5_7.
151. **Baeza J, Dowell JA, Smallegan MJ, Fan J, Amador-Noguez D, Khan Z, Denu JM.** 2014. Stoichiometry of site-specific lysine acetylation in an entire proteome. J Biol Chem **289:**21326–21338. http://dx.doi.org/10.1074/jbc.M114.581843.
152. **Kuhn ML, Zemaitaitis B, Hu LI, Sahu A, Sorensen D, Minasov G, Lima BP, Scholle M, Mrksich M, Anderson WF, Gibson BW, Schilling B, Wolfe AJ.** 2014. Structural, kinetic and proteomic characterization of acetyl phosphate-dependent bacterial protein acetylation. PLoS One **9:**e94816. http://dx.doi.org/10.1371/journal.pone.0094816.
153. **Wang Q, Zhang Y, Yang C, Xiong H, Lin Y, Yao J, Li H, Xie L, Zhao W, Yao Y, Ning ZB, Zeng R, Xiong Y, Guan KL, Zhao S, Zhao GP.** 2010. Acetylation of metabolic enzymes coordinates carbon source utilization and metabolic flux. Science **327:**1004–1007. http://dx.doi.org/10.1126/science.1179687.
154. **Kim D, Yu BJ, Kim JA, Lee YJ, Choi SG, Kang S, Pan JG.** 2013. The acetylproteome of Gram-positive model bacterium *Bacillus subtilis*. Proteomics **13:**1726–1736. http://dx.doi.org/10.1002/pmic.201200001.
155. **Wu X, Vellaichamy A, Wang D, Zamdborg L, Kelleher NL, Huber SC, Zhao Y.** 2013. Differential lysine acetylation profiles of *Erwinia amylovora* strains revealed by proteomics. J Proteomics **79:**60–71. http://dx.doi.org/10.1016/j.jprot.2012.12.001.
156. **Zhang Y, Wu ZX, Wan XL, Liu P, Zhang JB, Ye Y, Zhao YM, Tan MJ.** 2014. Comprehensive profiling of lysine acetylome in *Staphylococcus aureus*. Sci China Chem **57:**732–738. http://dx.doi.org/10.1007/s11426-014-5100-4.
157. **Lee DW, Kim DI, Lee YJ, Kim JA, Choi JY, Kang S, Pan JG.** 2013. Proteomic analysis of acetylation in thermophilic *Geobacillus kaustophilus*. Proteomics **13:**2278–2282. http://dx.doi.org/10.1002/pmic.201200072.
158. **Pan J, Ye Z, Cheng Z, Peng X, Wen L, Zhao F.** 2014. Systematic analysis of the lysine acetylome in *Vibrio parahemolyticus*. J Proteome Res **13:**3294–3302. http://dx.doi.org/10.1021/pr500133t.
159. **Okanishi H, Kim K, Masui R, Kuramitsu S.** 2013. Acetylome with structural mapping reveals the significance of lysine acetylation in *Thermus thermophilus*. J Proteome Res **12:**3952–3968. http://dx.doi.org/10.1021/pr400245k.
160. **van Noort V, Seebacher J, Bader S, Mohammed S, Vonkova I, Betts MJ, Kuhner S, Kumar R, Maier T, O'Flaherty M, Rybin V, Schmeisky A, Yus E, Stulke J, Serrano L, Russell RB, Heck AJ, Bork P, Gavin AC.** 2012. Cross-talk between phosphorylation and lysine acetylation in a genome-reduced bacterium. Mol Syst Biol **8:**571. http://dx.doi.org/10.1038/msb.2012.4.
161. **Liao G, Xie L, Li X, Cheng Z, Xie J.** 2014. Unexpected extensive lysine acetylation in the trump-card antibiotic producer *Streptomyces roseosporus* revealed by proteome-wide profiling. J Proteomics **106:**260–2699. http://dx.doi.org/10.1016/j.jprot.2014.04.017.
162. **Zhang K, Zheng S, Yang JS, Chen Y, Cheng Z.** 2013. Comprehensive profiling of protein lysine acetylation in Escherichia coli. J Proteome Res **12:**844–851. http://dx.doi.org/10.1021/pr300912q.
163. **Guan KL, Xiong Y.** 2011. Regulation of intermediary metabolism by protein acetylation. Trends Biochem Sci **36:**108–116. http://dx.doi.org/10.1016/j.tibs.2010.09.003.
164. **Karanam B, Jiang L, Wang L, Kelleher NL, Cole PA.** 2006. Kinetic and mass spectrometric analysis of p300 histone acetyltransferase domain autoacetylation. J Biol Chem **281:**40292–402301. http://dx.doi.org/10.1074/jbc.M608813200.
165. **Yang C, Wu J, Sinha SH, Neveu JM, Zheng YG.** 2012. Autoacetylation of the MYST lysine acetyltransferase MOF. J Biol Chem **287:**34917–34926. http://dx.doi.org/10.1074/jbc.M112.359356.
166. **Barak R, Yan J, Shainskaya A, Eisenbach M.** 2006. The chemotaxis response regulator CheY can catalyze its own acetylation. J Mol Biol **359:**251–265. http://dx.doi.org/10.1016/j.jmb.2006.03.033.
167. **Crosby HA, Escalante-Semerena JC.** 2014. The acetylation motif in AMP-forming acyl coenzyme A synthetases contains residues critical for acetylation and recognition by the protein acetyltransferase Pat of *Rhodopseudomonas palustris*. J Bacteriol **196:**1496–1504. http://dx.doi.org/10.1128/JB.00004-14.
168. **You D, Yao LL, Huang D, Escalante-Semerena JC, Ye BC.** 2014. Acetyl-CoA synthetase is acetylated on multiple lysine residues by a protein acetyltransferase with single GNAT domain in *Saccharopolyspora erythraea*. J Bacteriol **196:**3169–3178. http://dx.doi.org/10.1128/JB.01961-14.
169. **Starai VJ, Escalante-Semerena JC.** 2004. Acetyl-coenzyme A synthetase (AMP forming). Cell Mol Life Sci **61:**2020–2030.
170. **Karan D, David JR, Capy P.** 2001. Molecular evolution of the AMP-forming acetyl-CoA synthetase. Gene **265:**95–101. http://dx.doi.org/10.1016/S0378-1119(01)00358-4.
171. **Ingram-Smith C, Smith KS.** 2007. AMP-forming acetyl-CoA synthetases in Archaea show unexpected diversity in substrate utilization. Archaea **2:**95–107.
172. **Gulick AM.** 2009. Conformational dynamics in the acyl-CoA synthetases, adenylation domains of non-ribosomal peptide synthetases, and firefly luciferase. ACS Chem Biol **4:**811–827. http://dx.doi.org/10.1021/cb900156h.
173. **Brown TD, Jones-Mortimer MC, Kornberg HL.** 1977. The enzymic interconversion of acetate and acetyl-coenzyme A in *Escherichia coli*. J Gen Microbiol **102:**327–336. http://dx.doi.org/10.1099/00221287-102-2-327.
174. **Wu R, Cao J, Lu X, Reger AS, Gulick AM, Dunaway-Mariano D.** 2008. Mechanism of 4-chlorobenzoate:coenzyme A ligase catalysis. Biochemistry **47:**8026–8039. http://dx.doi.org/10.1021/bi800698m.
175. **Branchini BR, Murtiashaw MH, Magyar RA, Anderson SM.** 2000. The role of lysine 529, a conserved residue of the acyl-adenylate-forming enzyme superfamily, in firefly luciferase. Biochemistry **39:**5433–5440. http://dx.doi.org/10.1021/bi9928804.
176. **Hallows WC, Lee S, Denu JM.** 2006. Sirtuins deacetylate and activate mammalian acetyl-CoA synthetases. Proc Natl Acad Sci U S A **103:**10230–10235. http://dx.doi.org/10.1073/pnas.0604392103.
177. **Castano-Cerezo S, Bernal V, Blanco-Catala J, Iborra JL, Canovas M.** 2011. cAMP-CRP co-ordinates the expression of the protein acetylation pathway with central metabolism in *Escherichia coli*. Mol Microbiol **82:**1110–1128. http://dx.doi.org/10.1111/j.1365-2958.2011.07873.x.
178. **Xu H, Hegde SS, Blanchard JS.** 2011. Reversible acetylation and inactivation of *Mycobacterium tuberculosis* acetyl-CoA synthetase is dependent on cAMP. Biochemistry **50:**5883–5892. http://dx.doi.org/10.1021/bi200156t.
179. **Chan CH, Garrity J, Crosby HA, Escalante-Semerena JC.** 2011. In *Salmonella enterica*, the sirtuin-dependent protein acylation/deacylation system (SDPADS) maintains energy homeostasis during growth on low concentrations of acetate. Mol Microbiol **80:**168–183. http://dx.doi.org/10.1111/j.1365-2958.2011.07566.x.
180. **Lima BP, Antelmann H, Gronau K, Chi BK, Becher D, Brinsmade SR, Wolfe AJ.** 2011. Involvement of protein acetylation in glucose-induced transcription of a stress-responsive promoter. Mol Microbiol **81:**1190–1204. http://dx.doi.org/10.1111/j.1365-2958.2011.07742.x.
181. **Lima BP, Thanh Huyen TT, Basell K, Becher D, Antelmann H, Wolfe AJ.** 2012. Inhibition of acetyl phosphate-dependent transcription by an acetylatable lysine on RNA polymerase. J Biol Chem **287:**32147–32160. http://dx.doi.org/10.1074/jbc.M112.365502.
182. **Gardner JG, Escalante-Semerena JC.** 2009. In *Bacillus subtilis*, the sirtuin protein deacetylase, encoded by the *srtN* gene (formerly *yhdZ*), and functions encoded by the *acuABC* genes control the activity of acetyl coenzyme A synthetase. J Bacteriol **191:**1749–1755. http://dx.doi.org/10.1128/JB.01674-08.
183. **Grundy FJ, Waters DA, Takova TY, Henkin TM.** 1993. Identification of genes involved in utilization of acetate and acetoin in *Bacillus subtilis*. Mol Microbiol **10:**259–271. http://dx.doi.org/10.1111/j.1365-2958.1993.tb01952.x.
184. **Huang M, Oppermann-Sanio FB, Steinbuchel A.** 1999. Biochemical and molecular characterization of the *Bacillus subtilis* acetoin catabolic pathway. J Bacteriol **181:**3837–3841.
185. **Seow KT, Meurer G, Gerlitz M, Wendt-Pienkowski E, Hutchinson CR, Davies J.** 1997. A study of iterative type II polyketide synthases, using bacterial genes cloned from soil DNA: a means to access and use genes from uncultured microorganisms. J Bacteriol **179:**7360–7368.
186. **Courtois S, Cappellano CM, Ball M, Francou FX, Normand P, Helynck G, Martinez A, Kolvek SJ, Hopke J, Osburne MS, August PR, Nalin R, Guerineau M, Jeannin P, Simonet P, Pernodet JL.** 2003.

Recombinant environmental libraries provide access to microbial diversity for drug discovery from natural products. Appl Environ Microbiol **69:**49–55. http://dx.doi.org/10.1128/AEM.69.1.49-55.2003.
187. **McMahon MD, Guan C, Handelsman J, Thomas MG.** 2012. Metagenomic analysis of *Streptomyces lividans* reveals host-dependent functional expression. Appl Environ Microbiol **78:**3622–3629. http://dx.doi.org/10.1128/AEM.00044-12.
188. **Chater KF.** 2006. *Streptomyces* inside-out: a new perspective on the bacteria that provide us with antibiotics. Philos Trans R Soc Lond B Biol Sci **361:**761–768. http://dx.doi.org/10.1098/rstb.2005.1758.
189. **Mitchell CA, Tucker AC, Escalante-Semerena JC, Gulick AM.** 2015. The structure of S. lividans acetoacetyl-CoA synthetase shows a novel interaction between the C-terminal extension and the N-terminal domain. Proteins **83:**575–581. http://dx.doi.org/10.1002/prot.24738.
190. **Mikulik K, Felsberg J, Kudrnacova E, Bezouskova S, Setinova D, Stodulkova E, Zidkova J, Zidek V.** 2012. CobB1 deacetylase activity in *Streptomyces coelicolor*. Biochem Cell Biol **90:**179–187. http://dx.doi.org/10.1139/o11-086.
191. **Nambi S, Basu N, Visweswariah SS.** 2010. Cyclic AMP-regulated protein lysine acetylases in mycobacteria. J Biol Chem **285:**24313–24323. http://dx.doi.org/10.1074/jbc.M110.118398.
192. **Nambi S, Gupta K, Bhattacharyya M, Ramakrishnan P, Ravikumar V, Siddiqui N, Thomas AT, Visweswariah SS.** 2013. Cyclic AMP-dependent protein lysine acylation in mycobacteria regulates fatty acid and propionate metabolism. J Biol Chem **288:**14114–14124. http://dx.doi.org/10.1074/jbc.M113.463992.
193. **Nambi S, Badireddy S, Visweswariah SS, Anand GS.** 2012. Cyclic AMP-induced conformational changes in mycobacterial protein acetyltransferases. J Biol Chem **287:**18115–18129. http://dx.doi.org/10.1074/jbc.M111.328112.
194. **Lee HJ, Lang PT, Fortune SM, Sassetti CM, Alber T.** 2012. Cyclic AMP regulation of protein lysine acetylation in *Mycobacterium tuberculosis*. Nat Struct Mol Biol **19:**811–818. http://dx.doi.org/10.1038/nsmb.2318.
195. **O'Toole R, Williams HD.** 2003. Universal stress proteins and *Mycobacterium tuberculosis*. Res Microbiol **154:**387–392. http://dx.doi.org/10.1016/S0923-2508(03)00081-0.
196. **Escalante-Semerena JC.** 2010. N^{ϵ}-Lysine acetylation control conserved in all three domains of life. Microbe Mag **5:**340–344.
197. **Hase T, Wakabayashi S, Matsubara H, Kerscher L, Oesterhelt D, Rao KK, Hall DO.** 1978. Complete amino acid sequence of *Halobacterium halobium* ferredoxin containing an N epsilon-acetyllysine residue. J Biochem **83:**1657–1670.
198. **Sandman K, Reeve JN.** 2005. Archaeal chromatin proteins: different structures but common function? Curr Opin Microbiol **8:**656–661. http://dx.doi.org/10.1016/j.mib.2005.10.007.
199. **Forbes AJ, Patrie SM, Taylor GK, Kim YB, Jiang L, Kelleher NL.** 2004. Targeted analysis and discovery of posttranslational modifications in proteins from methanogenic archaea by top-down MS. Proc Natl Acad Sci U S A **101:**2678–2683. http://dx.doi.org/10.1073/pnas.0306575101.
200. **Bell SD, Botting CH, Wardleworth BN, Jackson SP, White MF.** 2002. The interaction of Alba, a conserved archaeal chromatin protein, with Sir2 and its regulation by acetylation. Science **296:**148–151. http://dx.doi.org/10.1126/science.1070506.
201. **Wardleworth BN, Russell RJ, Bell SD, Taylor GL, White MF.** 2002. Structure of Alba: an archaeal chromatin protein modulated by acetylation. EMBO J **21:**4654–4662. http://dx.doi.org/10.1093/emboj/cdf465.
202. **Guo R, Xue H, Huang L.** 2003. Ssh10b, a conserved thermophilic archaeal protein, binds RNA in vivo. Mol Microbiol **50:**1605–1615. http://dx.doi.org/10.1046/j.1365-2958.2003.03793.x.
203. **Majdalani N, Gottesman S.** 2005. The Rcs phosphorelay: a complex signal transduction system. Annu Rev Microbiol **59:**379–405. http://dx.doi.org/10.1146/annurev.micro.59.050405.101230.
204. **Huang YH, Ferrieres L, Clarke DJ.** 2006. The role of the Rcs phosphorelay in Enterobacteriaceae. Res Microbiol **157:**206–212. http://dx.doi.org/10.1016/j.resmic.2005.11.005.
205. **Hu LI, Chi BK, Kuhn ML, Filippova EV, Walker-Peddakotla AJ, Basell K, Becher D, Anderson WF, Antelmann H, Wolfe AJ.** 2013. Acetylation of the response regulator RcsB controls transcription from a small RNA promoter. J Bacteriol **195:**4174–4886. http://dx.doi.org/10.1128/JB.00383-13.
206. **Barak R, Prasad K, Shainskaya A, Wolfe AJ, Eisenbach M.** 2004. Acetylation of the chemotaxis response regulator CheY by acetyl-CoA synthetase purified from *Escherichia coli*. J Mol Biol **342:**383–401. http://dx.doi.org/10.1016/j.jmb.2004.07.020.
207. **Ramakrishnan R, Schuster M, Bourret RB.** 1998. Acetylation at Lys-92 enhances signaling by the chemotaxis response regulator protein CheY. Proc Natl Acad Sci U S A **95:**4918–4923. http://dx.doi.org/10.1073/pnas.95.9.4918.
208. **Liarzi O, Barak R, Bronner V, Dines M, Sagi Y, Shainskaya A, Eisenbach M.** 2010. Acetylation represses the binding of CheY to its target proteins. Mol Microbiol **76:**932–943. http://dx.doi.org/10.1111/j.1365-2958.2010.07148.x.
209. **Li R, Gu J, Chen YY, Xiao CL, Wang LW, Zhang ZP, Bi LJ, Wei HP, Wang XD, Deng JY, Zhang XE.** 2010. CobB regulates *Escherichia coli* chemotaxis by deacetylating the response regulator CheY. Mol Microbiol **76:**1162–1174. http://dx.doi.org/10.1111/j.1365-2958.2010.07125.x.
210. **Yan J, Barak R, Liarzi O, Shainskaya A, Eisenbach M.** 2008. In vivo acetylation of CheY, a response regulator in chemotaxis of *Escherichia coli*. J Mol Biol **376:**1260–1271. http://dx.doi.org/10.1016/j.jmb.2007.12.070.
211. **Barak R, Welch M, Yanovsky A, Oosawa K, Eisenbach M.** 1992. Acetyladenylate or its derivative acetylates the chemotaxis protein CheY in vitro and increases its activity at the flagellar switch. Biochemistry **31:**10099–11107. http://dx.doi.org/10.1021/bi00156a033.
212. **Fraser ME, James MN, Bridger WA, Wolodko WT.** 1999. A detailed structural description of Escherichia coli succinyl-CoA synthetase. J Mol Biol **285:**1633–1653. http://dx.doi.org/10.1006/jmbi.1998.2324.
213. **Tucker AC, Escalante-Semerena JC.** 2014. Determinants within the C-terminal domain of *Streptomyces lividans* acetyl-CoA synthetase that block acetylation of its active site lysine in vitro by the protein acetyltransferase (Pat) enzyme. PLoS One **9:**e99817. http://dx.doi.org/10.1371/journal.pone.0099817.
214. **Crosby HA, Rank KC, Rayment I, Escalante-Semerena JC.** 2012. Structure-guided expansion of the substrate range of methylmalonyl-CoA synthetase (MatB) of *Rhodopseudomonas palustris*. Appl Environ Microbiol **78:**6619–6629. http://dx.doi.org/10.1128/AEM.01733-12.
215. **Crosby HA, Rank KC, Rayment I, Escalante-Semerena JC.** 2012. Structural insights into the substrate specificity of the protein acetyltransferase *Rp*Pat: identification of a loop critical for recognition by *Rp*Pat. J Biol Chem **287:**41392–41404. http://dx.doi.org/10.1074/jbc.M112.417360.
216. **Bains J, Boulanger MJ.** 2007. Biochemical and structural characterization of the paralogous benzoate CoA ligases from *Burkholderia xenovorans* LB400: defining the entry point into the novel benzoate oxidation (box) pathway. J Mol Biol **373:**965–977. http://dx.doi.org/10.1016/j.jmb.2007.08.008.
217. **Bhaumik P, Koski MK, Glumoff T, Hiltunen JK, Wierenga RK.** 2005. Structural biology of the thioester-dependent degradation and synthesis of fatty acids. Curr Opin Struct Biol **15:**621–628. http://dx.doi.org/10.1016/j.sbi.2005.10.010.
218. **Skonberg C, Olsen J, Madsen KG, Hansen SH, Grillo MP.** 2008. Metabolic activation of carboxylic acids. Expert Opin Drug Metab Toxicol **4:**425–438. http://dx.doi.org/10.1517/17425255.4.4.425.
219. **Berg P.** 1956. Acyl adenylates: an enzymatic mechanism of acetate activation. J Biol Chem **222:**991–1013.
220. **Leonardi R, Zhang Y, Rock C, Jackowski S.** 2005. Coenzyme A: back in action. Prog Lipid Res **44:**125–153. http://dx.doi.org/10.1016/j.plipres.2005.04.001.
221. **Brass EP.** 1994. Overview of coenzyme A metabolism and its role in cellular toxicity. Chem Biol Interact **90:**203–214. http://dx.doi.org/10.1016/0009-2797(94)90010-8.
222. **El-Mansi M.** 2005. Free CoA-mediated regulation of intermediary and central metabolism: an hypothesis which accounts for the excretion of alpha-ketoglutarate during aerobic growth of *Escherichia coli* on acetate. Res Microbiol **156:**874–879. http://dx.doi.org/10.1016/j.resmic.2005.04.008.
223. **El-Mansi M, Cozzone AJ, Shiloach J, Eikmanns BJ.** 2006. Control of carbon flux through enzymes of central and intermediary metabolism during growth of *Escherichia coli* on acetate. Curr Opin Microbiol **9:**173–179. http://dx.doi.org/10.1016/j.mib.2006.02.002.
224. **Guest JR, Miles JS, Roberts RE, Woods SA.** 1985. The fumarase genes of Escherichia coli: location of the fumB gene and discovery of a new gene (fumC). J Gen Microbiol **131:**2971–2984.
225. **Guest JR.** 1979. Anaerobic growth of *Escherichia coli* K12 with fumarate

as terminal electron acceptor. Genetic studies with menaquinone and fluoroacetate-resistant mutants. J Gen Microbiol **115:**259–271.
226. **Izui K, Taguchi M, Morikawa M, Katsuki H.** 1981. Regulation of *Escherichia coli* phosphoenolpyruvate carboxylase by multiple effectors *in vivo*. II. Kinetic studies with a reaction system containing physiological concentrations of ligands. J Biochem **90:**1321–1331.
227. **Sanwal BD.** 1970. Allosteric controls of amphilbolic pathways in bacteria. Bacteriol Rev **34:**20–39.
228. **Vallari DS, Rock CO.** 1985. Pantothenate transport in *Escherichia coli*. J Bacteriol **162:**1156–1161.
229. **Vallari DS, Jackowski S.** 1988. Biosynthesis and degradation both contribute to the regulation of coenzyme A content in Escherichia coli. J Bacteriol **170:**3961–3966.
230. **Chohnan S, Furukawa H, Fujio T, Nishihara H, Takamura Y.** 1997. Changes in the size and composition of intracellular pools of nonesterified coenzyme A and coenzyme A thioesters in aerobic and facultatively anaerobic bacteria. Appl Environ Microbiol **63:**553–560.
231. **Chohnan S, Izawa H, Nishihara H, Takamura Y.** 1998. Changes in size of intracellular pools of coenzyme A and its thioesters in Escherichia coli K-12 cells to various carbon sources and stresses. Biosci Biotechnol Biochem **62:**1122–1128. http://dx.doi.org/10.1271/bbb.62.1122.
232. **Jackowski S, Rock CO.** 1986. Consequences of reduced intracellular coenzyme A content in *Escherichia coli*. J Bacteriol **166:**866–871.
233. **Keating DH, Carey MR, Cronan JE, Jr.** 1995. The unmodified (apo) form of *Escherichia coli* acyl carrier protein is a potent inhibitor of cell growth. J Biol Chem **270:**22229–22235. http://dx.doi.org/10.1074/jbc.270.38.22229.
234. **Keating DH, Zhang Y, Cronan JE, Jr.** 1996. The apparent coupling between synthesis and posttranslational modification of *Escherichia coli* acyl carrier protein is due to inhibition of amino acid biosynthesis. J Bacteriol **178:**2662–2667.
235. **Cahová H, Winz M, Hofer K, Nubel G, Jaschke A.** 2015. NAD captureSeq indicates NAD as a bacterial cap for a subset of regulatory RNAs. Nature **519:**374–377. http://dx.doi.org/10.1038/nature14020.
236. **Kolb A, Busby S, Buc H, Garges S, Adhya A.** 1993. Transcriptional regulation by cAMP and its receptor protein. Annu Rev Biochem **62:**749–795. http://dx.doi.org/10.1146/annurev.bi.62.070193.003533.
237. **Bai G, Schaak DD, McDonough KA.** 2009. cAMP levels within *Mycobacterium tuberculosis* and *Mycobacterium bovis* BCG increase upon infection of macrophages. FEMS Immunol Med Microbiol **55:**68–73. http://dx.doi.org/10.1111/j.1574-695X.2008.00500.x.
238. **Bai G, Knapp GS, McDonough KA.** 2011. Cyclic AMP signalling in mycobacteria: redirecting the conversation with a common currency. Cell Microbiol **13:**349–358. http://dx.doi.org/10.1111/j.1462-5822.2010.01562.x.
239. **Findeisen F, Linder JU, Schultz A, Schultz JE, Brugger B, Wieland F, Sinning I, Tews I.** 2007. The structure of the regulatory domain of the adenylyl cyclase Rv1264 from *Mycobacterium tuberculosis* with bound oleic acid. J Mol Biol **369:**1282–1295. http://dx.doi.org/10.1016/j.jmb.2007.04.013.
240. **Tews I, Findeisen F, Sinning I, Schultz A, Schultz JE, Linder JU.** 2005. The structure of a pH-sensing mycobacterial adenylyl cyclase holoenzyme. Science **308:**1020–1023. http://dx.doi.org/10.1126/science.1107642.
241. **Cann MJ, Hammer A, Zhou J, Kanacher T.** 2003. A defined subset of adenylyl cyclases is regulated by bicarbonate ion. J Biol Chem **278:**35033–35038. http://dx.doi.org/10.1074/jbc.M303025200.
242. **Abdel Motaal A, Tews I, Schultz JE, Linder JU.** 2006. Fatty acid regulation of adenylyl cyclase Rv2212 from *Mycobacterium tuberculosis* H37Rv. FEBS J **273:**4219–4228. http://dx.doi.org/10.1111/j.1742-4658.2006.05420.x.
243. **Baek SH, Li AH, Sassetti CM.** 2011. Metabolic regulation of mycobacterial growth and antibiotic sensitivity. PLoS Biol **9:**e1001065. http://dx.doi.org/10.1371/journal.pbio.1001065.
244. **Michan S, Sinclair D.** 2007. Sirtuins in mammals: insights into their biological function. Biochem J **404:**1–13. http://dx.doi.org/10.1042/BJ20070140.
245. **Ma Q, Wood TK.** 2011. Protein acetylation in prokaryotes increases stress resistance. Biochem Biophys Res Commun **410:**846–851. http://dx.doi.org/10.1016/j.bbrc.2011.06.076.
246. **Wolfe AJ.** 2005. The acetate switch. Microbiol Mol Biol Rev **69:**12–50. http://dx.doi.org/10.1128/MMBR.69.1.12-50.2005.
247. **Kumari S, Beatty CM, Browning DF, Busby SJ, Simel EJ, Hovel-Miner G, Wolfe AJ.** 2000. Regulation of acetyl coenzyme A synthetase in *Escherichia coli*. J Bacteriol **182:**4173–4179. http://dx.doi.org/10.1128/JB.182.15.4173-4179.2000.
248. **Browning DF, Beatty CM, Sanstad EA, Gunn KE, Busby SJ, Wolfe AJ.** 2004. Modulation of CRP-dependent transcription at the *Escherichia coli acs*P2 promoter by nucleoprotein complexes: anti-activation by the nucleoid proteins FIS and IHF. Mol Microbiol **51:**241–254.
249. **Tucker AC, Escalante-Semerena JC.** 2010. Biologically active isoforms of CobB sirtuin deacetylase in *Salmonella enterica* and *Erwinia amylovora*. J Bacteriol **192:**6200–6208. http://dx.doi.org/10.1128/JB.00874-10.
250. **Hulmes DJ.** 1992. The collagen superfamily—diverse structures and assemblies. Essays Biochem **27:**49–67.
251. **Errey JC, Blanchard JS.** 2006. Functional annotation and kinetic characterization of PhnO from *Salmonella enterica*. Biochemistry **45:**3033–3039. http://dx.doi.org/10.1021/bi052297p.
252. **Davies AM, Tata R, Beavil RL, Sutton BJ, Brown PR.** 2007. L-Methionine sulfoximine, but not phosphinothricin, is a substrate for an acetyltransferase (gene PA4866) from *Pseudomonas aeruginosa*: structural and functional studies. Biochemistry **46:**1829–1839. http://dx.doi.org/10.1021/bi0615238.
253. **Gutierrez A, Elez M, Clermont O, Denamur E, Matic I.** 2011. *Escherichia coli* YafP protein modulates DNA damaging property of the nitroaromatic compounds. Nucleic Acids Res **39:**4192–4201. http://dx.doi.org/10.1093/nar/gkr050.



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